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7S AND 19S ISOAGGLUTININS IN SYSTEMIC LUPUS ERYTHEMATOSUS*

By

JOHN BAUM**

Summary The 7S and 19S distribution of blood group isoantibodies (isoagglutinins) has been measured in patients with systemic lupus erythematosus. The only possibly significant finding was a lower level of macroglobulin isoagglutinin to B cells in patients of blood type A This is consistent with previous findings of lessened response to bacterial antigens by the macroglobulin antibody

INTRODUCTION

In recent studies we have found an apparent decrease in the production of macroglobulin antibody to specific bacterial antigens in *patients with systemic lupus erythematosus* (1) In order to continue our investigation of macroglobulin antibodies in SLE, we have studied isoagglutinin titers to A and B cells in a group of female patients with SLE and compared these with a pool of two control groups One was a group of student nurses The others were matched controls with pulmonary tuberculosis

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PATIENTS AND METHODS

Patients

There were 23 female patients with definite SLE All had posit L E cell preparations positive antinuclear fluorescence and had prese with various typical clinical features of the disease They ranged in from 14 to 56 (av 34) years The 18 in the student nurse control had an average age of 21 years (range 20—23) Eighteen matched trols with pulmonary tuberculosis were the same race age and sex the SLE patients to which they were matched None in any group known prior transfusions or injection of blood group substance

Methods

19S and 7S antibody titers were determined in serum fractions arated by sucrose density gradient centrifugation (5) Ten gradient tions were collected The 19S antibody titer was considered that ted from tube 3 and the 7S antibody titer that measured from t at the upper end of the gradient Localization of the immunogl was confirmed by several methods (1)

Isoagglutinin titers were determined by the method of Wegi Smithies using a microtitrator system (9) Fresh red blood cells pe A and B were obtained from the Parkland Hospital blood b each time the tests were run

Statistical analysis of the data was done using a program in Olivetti Underwood Programmer 101 for p values The significance then established with the use of the Student t test

RESULTS

Of the 23 patients with SLE 8 had anti A and anti B isoaggluti and were classed as type O There were 11 of type A (anti B i glutinins) 3 of type B (anti A isoagglutinins) and 1 AB (no glutinins to A or B type cells) The distribution of blood types in control groups was roughly the same (Table I) Type B was co in the SLE and Tbc group probably as a reflection of the number blacks in our population Type B is found in 20 percent of blacks co pared to 10 percent in the population (4) Because of the li ed number in each gr could be made for sta

TABLE I

	SLE patients	Controls	
		Student nurses	Tbc patients
Type 0	8	9	7
Type A	11	8	6
Type B	3	1	4
Type AB	1	0	1
	23	18	18

significance only with group 0 and A The control groups were pooled so that in group 0 the anti A and anti B titers could be compared In the type A group the anti B titers were compared

In the comparison of isoagglutinins to A cells no significant difference was noted between whole serum titers or between the titers measured in each of the separated gradient fractions indicating similar levels of *19S* and *7S* isoagglutinins Similar findings were noted when the same sera were compared for the isoagglutinin titers to type B cells However in the blood type A group it can be seen that there was a significantly lower level of macroglobulin isoantibody to B cells in the patients with systemic lupus (Fig 1)

Although the differences were not significant the SLE group in general showed lower titers of isoagglutinins in the whole serum to B cells in both group 0 and group A

DISCUSSION

While previous studies of isoagglutinin levels have been performed in patients with systemic lupus erythematosus (10) no investigations have been performed to determine the 7S and 19S distribution of the isoagglutinins In the study by Zingale and his colleagues (10) it was also noted that before stimulation with incompatible blood group substances the isoagglutinin titers were the same in the patients with SLE and in the control groups In another investigation by Muschel there was also no difference in whole serum levels of isoagglutinins between SLE patients and controls (7) As noted above we had similar findings of non significant differences between the SLE patients and the controls when whole serum isoagglutinin levels are compared

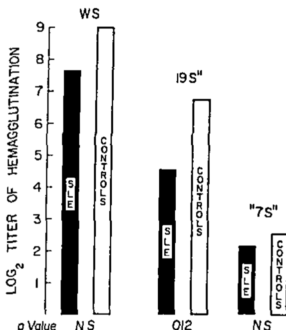


Fig 1 Isoagglutinin antibody levels to B cells in blood group A patients SLE and in controls Note the significantly lower titer of 19S antibody in patients with SLE

The isoagglutinins were compared separately in group 0 and gr A since it has been noted in another connective tissue disease *toid arthritis that there is some variation in the immunoglobulin di* bution of isoagglutinins in patients with different blood groups This study also found lower levels of isoagglutinins in patients rheumatoid arthritis than in controls though this was found onl group 0 individuals and not in groups A and B

Though isoagglutinin titers vary considerably between indivi they appear to be relatively constant in the same individual over ' *periods of time (6 3) nor are average levels affected by steroid* (8)

In previous studies we indicated a relative deficiency of 19S ant formation to bacterial antigens in patients with SLE (1) It has postulated that isoagglutinins arise by cross reactions with bacterial tigens (6) Though this response starts at an early age mainten of macroglobulin antibody is probably dependent on recurrent or sistent stimulation Any diminution in this response would then be

to a lack of or interference with antigenic stimulation or to a deficient response

Since the antigenic stimulation that maintains isoagglutinin levels is apparently universal it is unlikely that the former explanation for the lower titers would hold The restriction of the significantly lower titers to the macroglobulin antibody of the A group could be due to a deficiency in the response of this specific immunoglobulin to antigenic stimulation by bacterial antigens When the SLE patients were compared among themselves in those showing anti B isoagglutinins (in blood type A and 0) the only unusual feature was the fact that all the white patients with SLE were in group A No other parameter examined (age steroid doses gamma globulin level alpha 2 globulin level complement duration of disease renal disease or response to brucella antigen) was different in these two groups of patients with SLE (1)

Antigen competition can be considered as a possible mechanism for the diminished response The competition would be from the constant exposure to the large number of autoantigens (DNA erythrocytes leukocytes, thyroglobulin etc) to which a patient with lupus is frequently exposed and to which antibodies are continuously made (2)

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A DOUBLE BLIND TRIAL WITH CARTILAGE AND BONE MARROW EXTRACT* IN DEGENERATIVE GONARTHROSIS

By

E ADLER E WOLF and I TAUSTEIN

Summary A double blind trial with cartilage and bone marrow extra (Rumalon A) and placebo (Rumalon B) was carried out on 106 p tients with degenerative bilateral gonarthrosis Favorable results we obtained in 64 % of the patients treated with Rumalon A and in on 29 % of the placebo group This difference is statistically significa ($p < 0\ 05$) The slight influence of placebo can be related to the ps chosomatic effect of injections and/or coincident spontaneous improv ment of the gonarthrosis Cartilage and bone marrow extract increa the therapeutic possibilities in degenerative joint disease It is easy administer and practically without side effects

Degenerative osteoarthrosis is a non inflammatory disorder chara terized by degeneration of articular cartilage and formation of ne bone at the joint surfaces

An important role in the metabolism of the cartilage is played l chondroitin sulphate (2 3 4 9) which is responsible for the physi logical elasticity and solidity of the cartilage

Osteoarthrotic cartilage lesions show a decrease in chondroitin su phate concentration This decrease is proportional to the severity the osteoarthrotic lesions Collagen concentration of cartilage virtual

* "Rumalon" Robapharm Ltd Basel/Switzerland

remains unchanged (2 3) Collins and McElligott (7) demonstrated that human osteoarthrotic cartilage showed an increased uptake of radio sulphate in vitro when compared to normal cartilage from the same joints This uptake is presumably a measure of the rate of chondroitin sulphate synthesis suggesting greater synthesis in the areas which show decreased concentration of radiosulphate

Diagnosis of degenerative joint diseases is relatively easy but treatment is difficult Many kinds of treatment are recommended but their effect is mainly symptomatic and usually of very short duration

Rumalon is a new cartilage and bone marrow extract which activates the metabolism of chondroitin sulphate (1 16 17) In their experiments on rats Weigel and Jasinski (17) proved that the uptake of radioactive sulphate in rat cartilage is significantly increased by Rumalon

In a double blind test we compared the efficacy of Rumalon with a placebo in degenerative gonarthrosis

MATERIAL AND METHODS

106 patients (99 females and 7 males) with bilateral osteoarthrosis of the knee joints were treated in the out patient clinic with one of two substances labelled Rumalon A and B respectively All of the patients were over 46 years of age

55 patients received Rumalon A while 51 received B The dosage was the same as that recommended by the original investigators an initial intramuscular injection of 0 1 ml followed by 0 5 ml and then 1 0 ml three times a week until a total of 20 injections had been given

Patients were examined before and during treatment and for periods of 2 to 12 months afterwards The gonarthrosis was confirmed by clinical laboratory and x ray findings The patients had various stages of gonarthrosis (from moderate to mild) and were chosen at random when they visited our clinic

The response to treatment was assessed subjectively and objectively subjectively by questioning the patient with regard to pain at rest and during movement and to physical performance in general Objective criteria were local findings in the joints including swelling crepitation palpation of the periarticular tissue and especially measureable alterations in the degree of mobility of the knees

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* Rumalon Robapharm Ltd Basel/Switzerland

(b) good — considerable improvement for periods of up to five months but without complete disappearance of symptoms

(c) fair — slight improvement during treatment but further treatment needed immediately after termination of the series of injections

The number of patients in each group was too small for separate analysis but it is interesting to note that of the patients who received Rumalon A 6 had excellent 19 good and 4 fair results Of those who received the placebo none showed excellent results only three could be graded as good and the rest as fair

DISCUSSION

In this double blind trial 106 patients with different stages of gonarthrosis received cartilage and bone marrow extract (Rumalon A) or a placebo (Rumalon B)

64 % of the patients who received Rumalon A showed favorable results whereas only 29 % who received placebo Rumalon B reported some kind of improvement This difference is statistically significant ($p < 0\,05$) Flemmich (8) also found satisfactory results in about 80 % of 70 patients with osteoarthrosis Large joints in particular responded well to this treatment Wagenhauser (14) in a series of 144 patients found that the cartilage and bone marrow extract gave the best results in osteoarthrosis of the fingers (70 %) and the knees (62 %) In another trial with 151 patients (15) he obtained similar results as did Ruffie (12) in 135 patients Schiavetti and Bray (15) found favorable results in 71 of 371 patients with degenerative osteoarthrotic changes of the knees hips and spine

We had successful results with the placebo (Rumalon B) in 29 % of the patients In none of them however could the response have been graded excellent Most of the cases showed only slight improvement and this can be explained by the fact that osteoarthrosis sometimes shows spontaneous remissions and because injections sometimes have a psychosomatic influence on patients It is of the utmost importance that this type of treatment can be administered to out patients and has only minimal side effects such as digestive or autonomic nervous system disturbances sweating tach[illegible] headaches or as in one of our cases u[illegible] (5 6 10 [illegible]

TABLE I

Results of Treatment

Treatment	Improvement No	Improvement %	No improvement No	No improvement %	Total No	Total %
Rumalon A	29	64	16	36	45	
Rumalon B	12	29	29	71	41	

* 20 patients are not included
15 because of lack of follow up
1 developed urticaria
4 for technical reasons

TABLE II

Relationship between Results of Treatment and Severity of Gonarthrosis

Severity of gonarthrosis	Treatment	Improvement No	Improvement %	No improvement No	No improvement %	Total No	Total %
Medium	Rumalon A	4	67	2	33	6	
	Rumalon B	3	23	10	77	13	
Mild	Rumalon A	25	64	14	36	39	
	Rumalon B	9	32	19	68	28	

** 20 patients are not included (See footnote table I)

The results of our investigations indicate that the treatment w cartilage and bone marrow extract increases the therapeutic possibiliti in degenerative joint disease

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SYNOVECTOMY OF THE METATARSOPHALANGEAL JOINTS IN RHEUMATOID ARTHRITIS

By

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Summary Synovectomy of II V mtp joints was performed on 33 feet in 28 patients The average follow up time was 15 months The results were evaluated with respect to pain, radiographic changes, and the patients own estimate Radiographic appearances were compared with the unoperated joints in the other foot The radiographic changes were also correlated with changes in the general activity of the disease

At present synovectomy is the most frequently performed operation in the surgical treatment of patients with rheumatoid arthritis in the Rheumatism Foundation Hospital It is applicable to most of the joints of both upper and lower extremities although difficulty of access has limited its use at the shoulder and hip Even at these sites confirmation of its value in the more peripheral joints may lead to increased use

Metatarsophalangeal synovectomy although technically a straight forward operation has only recently come into vogue and we have been able to find only one report in the literature (Brattstrom 1968)

At this hospital metatarsophalangeal (mtp) synovectomies have been performed since 1966 To date some 60 feet have been operated on The indications for surgery have been pain with persistent synovitis of the mtp joint

The operative technique has been the same in every case A dorsal transverse incision is made from the 2nd to the 5th metatarsophalangeal

joints A longitudinal incision to either side of the extensor tendon will expose the bulging synovium This is removed easily from the dorsum of the joint By distracting the toe and using narrow rongeurs at least a partial synovectomy can be performed in the lateral and plantar parts of the joint If a hammer toe deformity is present the extensor tendon is lengthened

MATERIAL AND METHODS

Patients were included in the series if synovectomy had been performed on three or more joints in one or both feet and if the follow up period was at least six months Of the 31 patients fulfilling these criteria three were not available for examination and were excluded The 28 patients examined included five in whom both feet had been operated on so that a total of 35 feet were available for study

The follow up period ranged from six months to two years with an average of 15 months All but one patient fulfilled the American Rheumatism Association s criteria for definite rheumatoid arthritis The one exception was classed as having possible rheumatoid arthritis

The operation was performed mainly in early cases since in the advanced case joint resection has proved to be an excellent procedure and experience with other joints suggests that synovectomy gives the best results in the early stages of joint involvement

Because the indications for operation were pain and persistent synovitis and the purpose of the operation was to halt or at least delay the progression of local joint damage results were assessed under three headings (i) radiographic appearance of the joint (ii) local pain and (iii) the patients overall opinion of the value of the operation

However these data while taking into account the fate of the operated joints fail to allow for the degree of progression or remission of the disease generally in a particular patient We have therefore compared the radiographic appearance of the operated joints with those of the other foot and have also compared the hand radiograph at the time of operation with that taken at follow up In addition the general activity of the disease has been measured using the objective criteria of Calkin s activity tables

TABLE I

RESULTS

According to	Good	Fair	Poor
X-ray changes	21	6	6
Pain	22	5	6
Patients estimate	27	2	4

RESULTS

Table I shows the results evaluated with respect to pain radiographic changes and the patient s own estimate

The radiographic results were arrived at by assessing osteoporosis and erosions in each of the joints operated upon and giving these appearances values from zero (normal) to 3 (severely involved) on a subjective impression of their severity By subtracting the pre-operative from the post-operative value a figure was obtained whose sign indicates the direction and whose magnitude indicates the degree of radiological change in each joint

Pain was measured in three different ways (i) by palpation of each mtp joint separately (ii) by asking the patient to walk on tiptoe and (iii) by observing gait and walking ability

It can be seen that results on the whole are good With regard to both pain and radiographic changes four out of six cases are ated as good one out of six as fair and one out of six as poor (Table I) Improvement in radiographic appearance and pain usually occur together since increased use leads to decreased osteoporosis

On the basis of the patients subjective opinion results were still better 80 % being satisfied with the operation (Table I)

It is not sufficient however in judging the value of synovectomy in rheumatoid arthritis to consider the operated joint alone since local improvement can occur in the absence of surgical treatment if a general

TABLE II

Comparison of results with control

+	0	-	All
15	12	6	33

remission of the disease is occurring We have tried to allow for this in two different ways

Firstly we have compared the radiographic changes in the operated joints with the metatarsophalangeal joints of the other foot or in those cases where both feet have been operated on with the progress of disease in the metacarpophalangeal joints of the hands

Secondly we have correlated the radiographic changes in the operated joints with the general activity of the rheumatoid process

Table II gives the results obtained by the first of these methods of comparison The plus group consists of feet where the operated joints showed improvement with respect to the controls The zero group is where the operated joints either improved or were unchanged while the control joints also showed improvement or no change The minus group consists of feet in which the operated joints deteriorated whether the control joints stayed the same improved or got worse

As would be expected this type of controlled study gives less favourable results than an uncontrolled one However almost half the feet studied obtained clear benefit from the operation The zero group is surprisingly large This may be due to the period of study having been too short to allow improvement to show itself or to concomitant improvement in both control and operated joints It should be noted that we have not measured the degree of radiological change only its direction in terms of improvement lack of change or deterioration

We were interested to know whether changes in the activity of the disease generally affect the result of the operation Therefore we divided the patients into three groups (i) activity decreased (ii) activity unchanged (iii) activity increased and correlated the radiological appearance of the operated feet with these The results are shown in table III

Our series is a small one consisting of relatively mild cases (average activity on the Calkin scale was 10 6 the scale extending from 0 to 30) and follow up is short Despite these provisoes the table is interesting and



Fig. 1. a) II—V mtp joints of a patient pre operatively in x ray picture *b)* The same joints one year post operatively (third metatarsal head was partially resected and a synovectomy of II—V mtp joints performed.)

TABLE III

Comparison of results with changes of activity

		Good	Fair	Poor
Act decreased	22	16	5	1
Act. the same	4	4	0	0
Act increased	7	1	1	5

shows clearly that a general remission is an important factor for the production of good results and conversely an increase in the activity of the disease worsens the result Without wishing to draw far reaching conclusions we feel that our results show the value of control material in the evaluation of the results of synovectomy

CONCLUSIONS

(1) Following mtp synovectomy the results as shown radiographically by changes in osteoporosis and erosions were favourable in two-thirds of feet satisfactory in one sixth and poor in one sixth

(2) With regard to pain relief the results were similar to those shown by radiographic changes that is two-thirds were good one sixth satisfactory and one sixth poor

(3) Comparing radiographic appearance of the operated joints with the other foot we found the operation was valuable in half the cases in one third its value uncertain and in one fifth it failed to prevent deterioration

(4) When the *radiographic changes* were correlated with changes in the general activity of the disease during the period of observation we found that the result improved if the disease remitted and vice versa

REFERENCE

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PREVALENCE OF RHEUMATOID ARTHRITIS IN DENSELY AND THINLY POPULATED AREAS IN SWEDEN

By

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Summary The prevalence of rheumatoid arthritis in 1 287 persons living in densely populated areas and 1 287 persons living in thinly populated areas, matched as to sex age, occupation and geographical area, was compared The patient material was obtained in an epidemiological survey of rheumatoid arthritis (ARA definition) performed in Sweden in 1961 1 6 % of the inhabitants in densely populated areas and 2.5 % in thinly populated areas had rheumatoid arthritis (classical, definite, probable, possible) The differences were indicative but not significant Classical plus definite rheumatoid arthritis, however, showed exactly the same prevalences in the two populations (0.5 %) Probable plus possible rheumatoid arthritis was indicatively more prevalent in the thinly populated areas (2 0 %) than in the densely populated (1 1 %)

The aim of the present study was to find out if populations living in thinly populated areas showed different prevalences of rheumatoid arthritis from those living in densely populated areas Some reports in the literature claim that such differences in the prevalences exist (1 2 3 6 10, 11 13, 17) If there really are differences it is possible that they are due to environmental factors which have been proposed to play a role in the pathogenesis of rheumatoid arthritis i e climatic environment (3) social conditions (10) living in damp housing (8 9)

MATERIAL AND METHODS

The prevalence of rheumatoid arthritis among inhabitants in the county of Skaraborg in Sweden living in densely populated districts was compared with that of inhabitants randomly selected and matched according to sex age occupation and geographical area but living in thinly populated districts The matching made it possible to eliminate many important background factors such as age, sex occupation in the comparisons Differences in the ratios of rheumatoid arthritis in the two groups were tested with the chi square test level of significance 95 per cent

The patient material was obtained in a survey on the prevalence of rheumatoid arthritis performed in 1961 in the county of Skaraborg and is accounted for in detail in a special publication (5) Rheumatoid arthritis was diagnosed according to ARA criteria (7 14) Every person within the area passed naked or seminaked through an examination room where their joints were investigated Data from hospital and out patient records regarding the persons investigated were collected from hospitals in which those with rheumatoid arthritis had been treated The diagnosis rheumatoid arthritis was based on a history of the disease macromorphological objective findings from the joints muscles and tendons and completed with available data from the hospital records and x ray documents

Definitions of a densely populated area more than 200 persons living per square km a thinly populated area less than 200 persons living per square km (4)

By aid of the population registers in Sweden it was exactly known whether a person lived in a densely or a thinly populated area (4) Registers of the population in Sweden are prepared through regular registrations for census purposes viz mantalsskrivning performed in the provincial civil registration offices As the last population census was performed in 1961 this register was absolutely up to date at the beginning of the investigation

RESULTS

Of 1 287 persons living in the densely populated area 21 (1 6 %) had rheumatoid arthritis (2 classical 5 definite 4 probable 10 possible) and of 1 287 matched persons living in the thinly populated area 52

(2.5 %) had rheumatoid arthritis (1 classical, 6 definite, 9 probable, 16 possible).

These differences were not considered significant (Chi 2 = 2.77) but indicative (90 %, one tailed probability). For classical and definite rheumatoid arthritis added no difference in prevalences exist between densely and thinly populated areas. For probable and possible rheumatoid arthritis added the prevalence of the disease is indicatively higher in thinly populated areas (Chi 2 = 3.0).

DISCUSSION

In some reports in the literature significantly higher prevalences of rheumatoid arthritis in rural than in urban districts have been observed (10, 13, 17). In one investigation (1) men in rural areas had a significantly higher rate for rheumatic diseases than those in urban areas: 2.7 % for males in urban and 2.4 % for males in rural districts, for females 6.8 % and 6.9 % respectively. The prevalence of rheumatoid arthritis in towns was reported to be higher than that in rural areas in Finland (6) in one investigation but another study (16) based on the statistics of the Central Office of Statistics of Finland (1950) revealed no significant differences between cities, towns and rural communities regarding the prevalence of rheumatoid arthritis. In one report no urban-rural difference was observed among patients aged 15—54 but in the age group 55 and older the urban prevalence was 1.3 % and the rural 3.0 % (12, 15). Rheumatoid arthritis in a Swedish investigation 1913—1915 was reported to be commoner and more malignant in the coastal districts than in the inland districts but this difference was more pronounced in the rural districts than in the villages (2, 3). One statistically significant urban-rural difference was noted where the prevalence of positive serological tests in the urban areas was 5 % against 2 % in the rural areas (11).

However, in these reports from the literature no careful matching of the inhabitants living in urban and rural districts was performed and no strict definition of urban and rural areas made. Thus the results from the literature are not comparable in all respects with the present investigation. It is possible that if the investigated populations had been larger the indicatively higher prevalences of probable and possible rheumatoid arthritis observed in thinly populated areas might have been significant.

Differences must be due to factors other than occupation age and sex which are eliminated in the present comparisons

It is important however that classical plus definite rheumatoid arthritis showed exactly the same prevalences in densely and thinly populated areas and the main conclusion of the investigation seems to be that no certain differences exist

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ARTHRITIS INDUCED WITH FREUND S ADJUVANT AND ITS RELATIONSHIP TO CHANGES IN THE STOMACH SMALL INTESTINE AND THYMUS

A Histological and Tritiated Thymidine Study on Rats

By

HELJO JULKUNEN and PENTTI ROKKANEN

Summary The aim of the study was to elucidate histological changes provoked by Mycobacterium butyricum toxine in the rat joints, stomach small intestine and thymus and the uptake of tritiated thymidine Freund s complete adjuvant was injected into the base of the vastus muscle of 35 rats 21 rats served as controls Fifteen rats displayed histologically *mild synovitis in the ankle joint, two periostitis and three muscle in* filtrates. Histological study revealed no difference between the test animals and the controls in the stomach small intestine and thymus The thymidine uptake of the adjacent growth lines of the knee stomach mucosa small intestine, spleen and liver was the same in both groups. In contrast the uptake of the cells of the thymus was smaller in the adjuvant treated rats with a probability of over 90 per cent This suggests that the mitosis of adjuvant induced thymic cells is smaller than in cells with a normal condition

Adjuvant arthritis in rats was used as the experimental model for rheumatoid arthritis Most rats develop either transient or lasting poly arthritis in the peripheral joints tail and base of the spinal column 10—20 days after the injection (in oil) of Mycobacterium butyricum or human Mycobacterium The arthritis may even cause fibrous or bony ankylosis Inflammatory changes have been demonstrated also in the

periosteum and the iris It is possible to transmit the disease to healthy rats by injecting viable cells from the lymph glands lymph fluid or spleen but it has not been produced by thymic cells (1 3 6 8 10 12)

We studied the changes in various joints the ventricular and intestinal mucosa and the thymus of rats after the injection of Freund s adjuvant both histologically and by measuring the uptake of tritiated thymidine

MATERIAL AND METHODS

The material consisted of 56 female Wistar rats aged three months and weighing 200—250 g In a pilot study 10 rats were injected subcutaneously in the tail with 1 mg of Mycobact butyricum/1 ml oil mixture (Complete Adjuvant Freund Difco 0638) No systemic poly arthritis was seen during the observation period of one month However ulceration of the site of injection was observed The 35 rats constituting the actual experimental series were therefore given 1 ml of the adjuvant intramuscularly in the base of the thigh Twenty-one rats served as controls The animals were distributed into seven groups each of five rats given adjuvant and three controls The rats were sacrificed 5 10 15 20 25 30 and 60 days after the injection All the test animals were given 0 2 mCi/g of body weight of H^3 thymidine (Radiochemical Centre Amersham England) 48 hours before they were decapitated Histological specimens were taken from the ankle and knee joints of every rat from the sacro-iliac joint and spine of 24 rats and from the stomach small intestine and thymus of every rat The bone and joint specimens were decalcified by the EDTA method All the specimens were stained with hematoxylin eosin and Alcian blue Contact autoradiography (9) with Kodak AR 10 film was done on all the organ and bone joint specimens The exposure time was about eight weeks The specimens were stained with Kern Echt Rot

RESULTS

Histological changes Nine of the 35 rats given the adjuvant displayed persistent thickening in the ankle joint in addition to edema of the injection site at the base of the thigh No generalised arthritis was established Histological examination revealed proliferation of synovial cells in

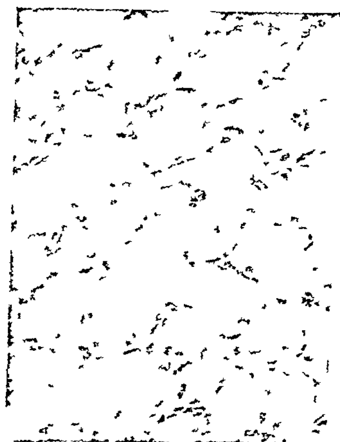


Fig. 1. Mucosa of the ventricle. Thymidine uptake by the undifferentiated cells of the neck region. 400x

the ankle joint of 15 rats. It was observed in one of the controls. No cartilage or bone destruction was seen, nor were any changes demonstrated in the sacroiliac and spinal joints. Periosteal cellular infiltration was demonstrated in the ankle of two rats and muscle infiltrates in three rats. No definite differences were seen in the organ specimens. The inflammatory changes in the gastric mucosa and the abundance of lymphatic tissue were evaluated blindly without the examiner knowing which of the pair of rats under examination had been given the adjuvant. The results of the histological study of the stomach are illustrated in fig. 3 (Sequential Analysis Chart, Geigy) which shows that no statistically significant differences were observed.

Autoradiographic changes. Uptake of tritiated thymidine by the joints



Fig 2 Cells of the epiphysial line marked by H³ thymidine 400 x

and the cells of the stomach small intestine spleen liver and thymus was studied The appearance of at least five marked points near the nucleus of a cell was taken to indicate uptake (Figs 1 2)

There was very rare uptake by the cells of the articular cartilage A growth line was seen in the femoral and tibial epiphysis of all the rats and there was uptake of thymidine by these cells The number of marked cells in the epiphysis of 18 rats given adjuvant and 14 control rats was counted per 0 025 mm measuring unit in the field of vision of the microscope The mean for the treated rats was 2 6 (0 2—5 3) cells and for the controls 2 1 (0 1—5 7) cells Histologically verified arthritis did not affect the number of marked cells

The uptake of thymidine by the gastric mucosal cells was evaluated by pairs in 22 pairs of rats using one control twice Six pairs displayed

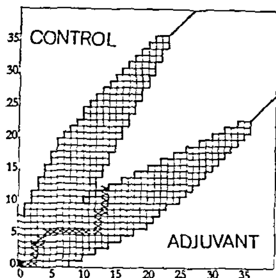


Fig 3 Sequential analysis of the inflammatory changes of the stomach in 25 pairs of adjuvant control rats Four controls were used twice

a similar eight a greater and eight a smaller uptake in the adjuvant group Hence no difference between the series was observed

Seventeen pairs of the splenic specimens were examined Five of them revealed a similar profuse uptake in the adjuvant group and the controls In six pairs the uptake was greater and in another six it was smaller in the treated animals

The uptake of the liver cells was slight and no difference was observed between the groups

Marked cells in the thymus were most numerous in the medulla The cells were counted per microscopic measuring field of 0 06 sq mm moving from one edge of the autoradiography sample to the other The average number of marked cells per field was 0 6 in the adjuvant group and 2 0 in the control group (Table I) Sequential analysis showed the difference to be significant with a probability of over 90 per cent (Fig 4)

DISCUSSION

Adjuvant induced arthritis has been regarded in many connections as an experimental model of *rheumatoid arthritis* because of the great sim

TABLE I

H^3 thymidine marked Cells in the Thymus per Field of 0 06 sq mm in the Adjuvant and Control Rats

	Number of rats	Mean number of marked cells	Range
Adjuvant	2?	0 6	0 1—3 ?
Control	13	2 0	0 2—6 ?

ilarity of the systemic reaction This reaction has been provoked by dead Mycobacteria and it is possible to transmit the disease with lymphocytes into healthy animals Even this does not necessarily imply an autoimmune disease as the phagocytosed bacterial products of adjuvant induced arthritis may be removed with the cells (5) Hence adjuvant induced arthritis cannot be regarded as a model at least not a good one of rheumatoid arthritis The adjuvant administered in the present study provoked arthritis fairly rarely in the rat strain employed and when it was induced it was fairly mild

It has been shown earlier that gastritis like histological changes are encountered in man in connection with connective tissue disease in a greater number of patients than controls (11) We tried to reproduce this phenomenon in rats by giving them adjuvant but we could not establish a reliable higher frequency of inflammatory changes in the gastric mucosa and proliferation of lymphatic tissue in them than in the controls although the tendency was discernible (Fig 3) The uptake of tritiated thymidine was also of the same level in both groups It does not seem consequently that mitosis of cells of the gastric mucosa is accelerated after the administration of an adjuvant

Thymectomy performed on newborn mice inhibited the development of lymphatic tissue The lymph glands and the spleen remained smaller than normal and scanty plasma cells were encountered (7) Thymectomy performed on rats aged five weeks did not inhibit the development of adjuvant induced polyarthritis (10) A decrease in the weight of the thymus was observed in conjunction with adjuvant induced arthritis though no histological changes were established (2) The smaller uptake of tritiated thymidine observed by us in the rats given adjuvant than in the control rats (Fig 4) also suggests that the activity of the thymus in the form of cell mitosis seems to be decreased after the administration

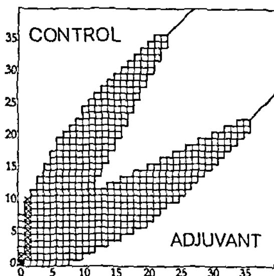


Fig 4 Sequential analysis of H^3 thymidine marked cells per field of the thymus in pairs of adjuvant and control rats

of adjuvant The difference cannot be accounted for even by the possible uptake of thymidine by the actively dividing inflammatory tissue in the adjuvant rats as the other organs displayed practically the same uptake of thymidine as in the controls

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SOME ASPECTS OF EPIDEMIOLOGY AND SURVEILLANCE OF RHEUMATIC FEVER

By

STEFAN SITAJ TIBOR URBANEK and KAROL BOSMANSKY

Summary The aim of the study was to find the most reliable and precise incidence of rheumatic fever attacks in the general population All new cases of rheumatic fever were recorded in an area of 100 000 population The frequency of first attacks and recurrences decreased from 53 per 100 000 inhabitants to 15 in 1968 Less pronounced was the fall in the incidence of first attacks The prevalence of rheumatic heart diseases in the studied group of patients was relatively low

Epidemiological studies are one of the most important components of the surveillance of rheumatic fever Further important tasks are early diagnosis and hospitalization introduction of adequate therapy and (effective) prophylactic measures with periodic checkups

A marked decrease in the incidence of rheumatic fever has been reported in recent years from the socio economically most advanced countries (1 7 9 10 12 15) Rheumatic fever and severe rheumatic heart diseases are also quite frequent in many tropical and subtropical areas (20)

Population morbidity data are scanty since in most countries rheumatic fever is not a reportable disease Moreover difficulties as regards the standardization of diagnostic criteria have led either to over diagnosis or to under diagnosis of rheumatic fever (5 7 8 10 13 11 18) The aim of the present study was to find the most reliable and precise figures for frequency of first attacks and recurrence of rheumatic fever in the general population

MATERIAL AND METHODS

All new cases of rheumatic fever were recorded in a selected area of 100 000 population in cooperation between the epidemiological group of the Research Institute of Rheumatic Diseases in Piestany and district physicians general practitioners pediatricians and internists Fig 1 indicates the area under observation with the network of health service centers There are 22 district physicians and two policlinics

The general practitioners were asked to send each patient showing the least symptoms or signs of rheumatic fever to the Research Institute where the members of the epidemiologic group by means of every accessible method of investigation either confirmed or eliminated the diagnosis of rheumatic fever The modified Jones diagnostic criteria have been used for the diagnosis (15 20) These criteria are not meant to replace the judgement of the clinician they are designed only to guide him towards the diagnosis of rheumatic fever Jones criteria have recently been re evaluated and the diagnostic importance of preceding beta hemolytic group A streptococcal infections has been stressed as in most cases the diagnosis of these infections can be made reliably by means of bacteriological and serological methods in combination with clinical findings (9 10 12 16 17)

RESULTS

The epidemiological study was started in 1961 and for technical reasons it was limited during the first two years to the age group of 15 years and over In subsequent years — from 1963 onwards — the child population up to 14 years was also included Fig 2 shows the incidence of first attacks and recurrences from 1961 to 1968 in a sample area of 100 000 inhabitants An unequivocal decrease of the frequency of attack rates especially in the population from 15 years and over is evident (see lower part of fig 2) There is a more pronounced decrease of recurrent attacks their number dropping from 37 1 in 1961 to 5 7 in 1968 i e to approximately one seventh The incidence of first attacks dropped from 25 7 in 1961 to 10 0 in 1968 i e to nearly one third In the child population the situation is somewhat different (upper part of the fig) The frequency of recurrent attacks dropped to zero the incidence of first attacks was reduced from 40 0 in 1963 to 13 3 in 19[illegible]

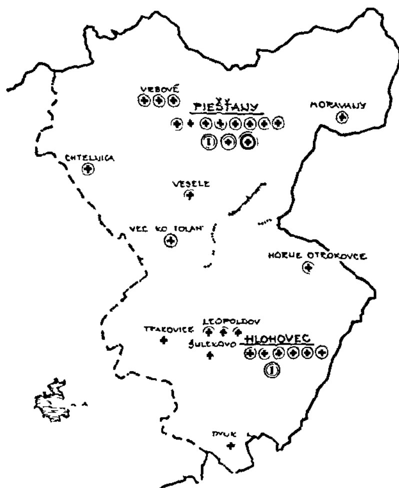


FIG 1 DISTRICT WITH 100000 INHABITANTS

- ⊕ HEALTH CENTRES
- ⊙ HOSPITAL
- ⊙ RESEARCH INSTITUTE OF RHEUMATIC DISEASES

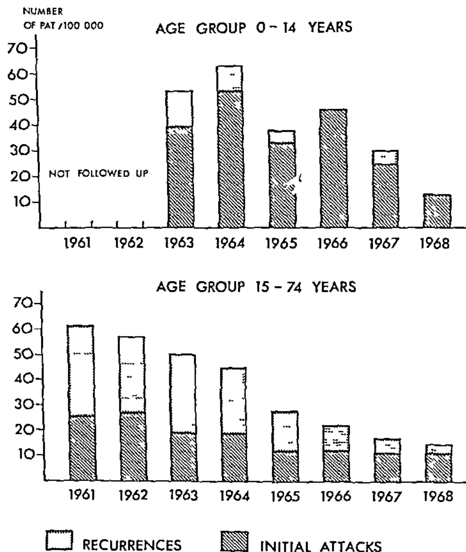


Fig 2 Incidence of rheumatic fever in 100 000 inhabitants from 1961 to 1968

The incidence of attacks in the whole population in the selected area can be seen in fig 3 The frequency of first attacks and recurrences dropped from 53 per 100 000 inhabitants in 1963 to 15 in 1968 This is a confirmation of data obtained by less exact observations (i e a

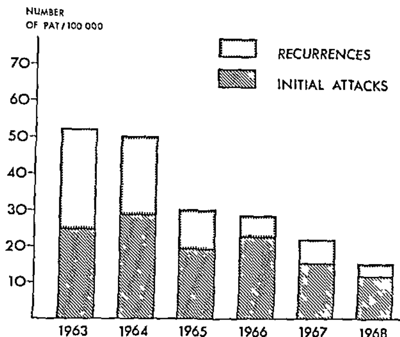


Fig 3 Incidence of rheumatic fever in 100 000 inhabitants from 1963 to 1968

decrease of requests for hospital admission) by some of the figures gained by notification of rheumatic fever as well as by post mortem examinations of the younger age groups where we also find a decreasing trend of rheumatic fever For instance in a Prague district the number of children hospitalized for rheumatic fever attacks dropped to one tenth (11) in the period from 1956 to 1963 in Denmark the number of notified cases dropped from 0 30 per mille (1955) to 0 12 per mille (1962) (2 4)

When ascertaining the age specific incidence of rheumatic fever attacks we found the highest rate of recurrent attacks in the age group 15—19 years, the highest incidence of first attacks was in the age group 10—11 years (Table I) The incidence of first attacks and recurrences in higher age groups is not negligible however

The absolute number of patients with definite rheumatic fever attacks in the epidemiological study in the years 1961—1968 amounted to 277 (first attacks 155 recurrences 122)

An analysis of the patients according to the incidence of individual Jones criteria shows that from the group of major criteria polyarthritis

TABLE I

Age Specific Incidence of Rheumatic Fever Attacks in 1963—1965

Age group	Number of inhabitants in the area	Number of attacks 1963—1965			Specific incidence per 100 000 inhabitants		
		First attacks	Recurrences	Total	First attacks	Recurrences	Total
0— 4	9 270	1	—	1	3 6	—	3 6
5— 9	9 797	17	3	20	57 6	10 8	67 8
10—14	10 549	20	5	25	63 2	15 8	79 0
15—19	9 788	9	15	24	36 6	51 0	87 6
20—24	8 191	7	6	13	28 5	24 4	52 9
25—34	13 410	8	8	16	19 8	19 8	39 6
35—44	13 607	8	15	23	19 6	36 7	56 3
45—54	9 834	—	3	3	—	10 2	10 2
55—64	10 358	3	5	8	9 6	16 1	25 7
65—74	6 157	—	1	1	—	5 4	5 4
75+	7 573	—	—	—	—	—	—
Total	103 484	73	61	134	23 5	19 6	43 1

and carditis are the most frequent (Fig 4) The incidence of three further criteria is negligible because of very low frequency despite their being characteristic signs of rheumatic fever

In the group of minor criteria the most frequent symptoms are fever (including subfebrility) and increased value of acute phase reactans (ESR CRP) These are present in practically all cases and qualify the attack as an acute illness An important finding is the evidence of preceding group A streptococcal disease in 88 per cent of the cases A detailed analysis of 202 attacks registered in the years 1961—1965 shows that only in 137 i e in 64 per cent was the initial streptococcal infection *clinically manifest and therefore liable to treatment* in a further 41 patients i e in 20 2 per cent the streptococcal infection was proved only serologically and was clinically symptom less These findings confirm the problems we meet with in the effective surveillance of group A streptococcal infection in connection with rheumatic fever The clinical symptoms and signs of streptococcal disease of the upper respiratory tract may vary from typical to completely atypical and therefore it often happens that the patient does not consult a physician On the other hand the physician in cases having less pronounced clinical manifestations may not assume the presence of streptococcal infection and may not prescribe

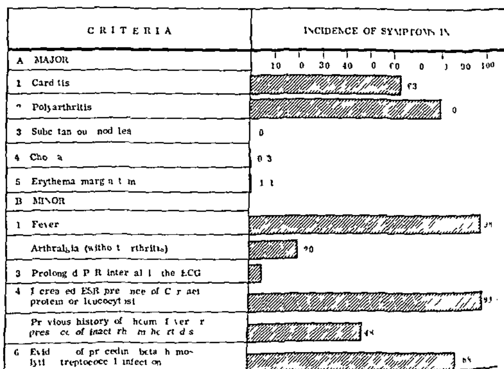


Fig 4 Incidence of diagnostic criteria according to Jones in 262 patients with rheumatic fever

adequate penicillin therapy or else gives other medication (3 6 10 17 18 19)

An attempt was made to evaluate the frequency of rheumatic heart diseases in the group of patients with first attacks of rheumatic fever in the period 1961—1965 i e with a time lapse of at least two years Definite rheumatic heart disease was diagnosed in 2 8 per cent in a further 5 per cent the disease was only probable

In the group of 202 patients treated in the years 1961—1965 continuous penicillin prophylaxis was not introduced in 21 cases for various technical reasons In 181 patients Pendepon comp Spofa (Benzathinum penicillinum 1 200 000 u Procainum pen G 300 000 u) was administered at monthly intervals As in the course of five years the patients were grouped gradually into the prophylaxis programme the duration of the follow up was different for each patient Therefore the index patient years was used in the evaluation of results

In a group of 181 patients under continuous penicillin prophylaxis the index was 388 4 On the contrary the total period when penicillin prophylaxis was not applied represents an index of 180 patient years There were two recurrences during continuous penicillin prophylaxis (total of 388 4 patient years i e 0 52 per cent) in patients unprotected by prophylaxis eight recurrences were ascertained (total of 180 5 patient years i e 4 4 per cent) This difference gives a clear evidence of the importance of penicillin prophylaxis in rheumatic fever Similar results were presented by others (6 7 8 10 16 17 18)

DISCUSSION

Epidemiological studies of rheumatic fever aimed at the incidence of first and recurrent attacks are one of the most important components of the surveillance of this disease Further important aspects of surveillance are

- early hospitalization of patients
- introduction of adequate anti infection (penicillin) and anti inflammatory therapy (salicylates glucocorticoids and long term prophylaxis (continuous administration of penicillin)
- periodic follow up examination of patients and finally
- nosographic analysis of rheumatic fever and chronic rheumatic heart disease which enable the doctor to register changes in the natural history and course of the disease

It is probable that prompt hospitalization due to early diagnosis and the introduction of appropriate treatment have a favorable effect on the course of rheumatic fever and on the evolution of rheumatic heart disease

The decrease in the number of recurrent attacks in the whole population can partly be attributed to long term strict surveillance of prophylactic measures partly to the possible effect of host factors on the occurrence of streptococcal infections and certainly also to some factors so far unknown

In so far as the incidence of first attacks is concerned the chief problem remains the small decrease of attacks in the child population With regard to our limited possibilities in the prevention of first attacks heightened attention should be paid to this problem in the future

The relatively low prevalence of chronic rheumatic heart diseases in this group of patients can be explained as follows

1) A generally more benign character of rheumatic fever attacks especially of carditis in the last two decades mainly in developed countries
2) Strict observance of surveillance of rheumatic fever in the group of patients followed up early diagnosis and hospitalization adequate and sufficiently long treatment continuous penicillin prophylaxis by means of special control cards, periodic check up of all patients
3) The follow up group of patients in the years 1961—1968 included all patients in the sample area therefore also light cases and thus it cannot be compared with selected clinical and hospital cases where a higher incidence of cardiopathies is probable

In principle the time lapse of 5—7 years after the end of the attacks is not a sufficiently long interval for making a definite assessment of heart damage in the individual patient The relatively low prevalence of chronic rheumatic heart disease in prospective follow up studies shows a certain discrepancy with current clinical experience over the past years in that the amount of rheumatic heart diseases decreased only to a limited extent In this connection however it may be mentioned that the present day increased possibilities in conservative treatment and cardio surgery prolong the life expectancy of cardiac patients

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INVOLVEMENT OF THE MANUBRIO STERNAL ARTICULATION IN RHEUMATOID ARTHRITIS

By

H LAITINEN S SAKSANEN and H SUORANTA

Summary Changes in the manubrio sternal articulation (MSA) were examined radiologically in 87 patients with rheumatoid arthritis and in a control group comprised of 23 patients with osteoarthritis and 72 subjects without joint disease Erosions reactive sclerosis and ankylosis were encountered in 70 % of the rheumatoid arthritis group and in 27 % of controls There was no difference in the incidence of changes in persons with osteoarthritis and in the other control subjects

Inflammatory changes in the amphiarthrodial articulations are considered characteristic of ankylosing spondylitis There is radiological evidence that such changes may occur not only in the sacro iliac joints but also in the manubrio sternal articulation (MSA) Erosions and destruction with osteoporosis have been encountered as signs of an active inflammatory process (1 3 5 6) Signs pointing chiefly to a degenerative process or a post inflammation state are unevenness and sclerosis of the joint surfaces and more or less complete fusion of the joint space (7 8)

In rheumatoid arthritis the diarthrodial joints bear the brunt of damage and the involvement of the amphiarthrodial joints in this disease is regarded as non typical Concerning the MSA information on its involvement in rheumatoid arthritis is very scanty probably partly because this joint is commonly overlooked clinically Only a few sporadic observations have been reported of changes in this articulation in RA Savill s series (7) includes 23 patients with RA in four of whom there

was narrowing of the joint cartilage and in five incomplete or complete fusion Dilsen et al (4) in their comparative radiological study of rheumatoid arthritis and ankylosing spondylitis concluded that the MSA may be affected in both diseases but that the frequency and degree of damage was much greater in ankylosing spondylitis Involvement was encountered in ankylosing spondylitis in 87 % of cases and in rheumatoid arthritis in 58 % and in non rheumatic controls there also was damage in 28 %

In the experience of the present writers changes in the MSA are not infrequent in rheumatoid arthritis The present study was undertaken with the object to obtain information on the incidence and radiological signs of these lesions

MATERIAL, METHOD AND RESULTS

The series comprised 87 cases of classical definite or probable rheumatoid arthritis The control group consisted of 95 hospital patients 23 of whom had osteoarthritis and 72 were subjects not suffering from any joint disease

The mean age of the rheumatoid group was 51 2 years of the osteoarthritis patients 59 9 years and of the other controls 48 3 years

The MSA was investigated by tomography in an anteroposterior and a direct lateral projection Often the changes found were also visible in the conventional lateral projection The following features were assessed in the radiographs osteoporosis joint space changes erosions reactive sclerosis and fusion and they were graded 0 + and ++ according to absent slight or marked changes On the basis of the principal finding the cases were distributed into groups as follows

Normal As normal variations were regarded slight osteoporosis a small change in the width of the joint space mild sclerosis at the bone margins and uneven joint surfaces

Erosions Fresh erosions were encountered with indistinct margins and associated osteoporosis (Fig 1) Erosions bordered by calcium seams were apparently signs of an old process (Fig 2) Frequently the erosions were situated in both articular surfaces the joint space resembling a string of beads (Fig 3)

Reactive sclerosis This change was characterized by a calcified zon

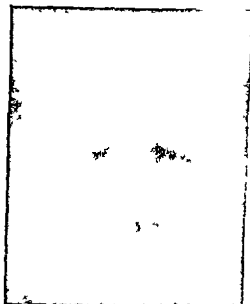


Fig 1 Female 22 yrs Probable RA for 8 months In the peripheral jo nts osteo poros s rad olog cally without destructive changes (Ste nbrocker s gr I) In the MSA erosions and localized marg nal scleros s Irregular ty of the jo nt space

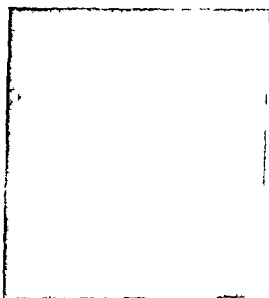


Fig 2 Male 30 yrs Pr bable RA f r 1 m nths N def n e x ray hanges in the per pheral jo nts (gr I) The MSA sh ws w den ng of j nt pace and in both surfaces er s ns surr unded by a calc f ed b ne seam

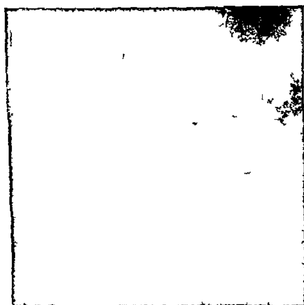


Fig 3 Female 40 yrs with clinically and radiologically advanced RA Erosions and marginal sclerosis in the MSA Initial ankylosis with intra articular calcified tissue

adjacent to eroded joint surfaces (Fig 4) Osteophytes were absent or very few

Ankylosis Two types of ankylosis were distinguished In one type the bone structure was homogeneous throughout and thus not even the site of the joint space could be detected We consider that this corresponds to the early primary synostosis described by Ashley (2) In the other type traces of the joint space were visible and the adjacent bone showed an irregular bone structure (Fig 4) calcifications and frequently small cyst like cavities (Fig 5) this being according to Ashley secondary or sclerotic synostosis

Table I presents the incidence and nature of the observed radiological changes

DISCUSSION

This series of cases domonstrates that changes in the MSA are remarkably often encountered in rheumatoid arthritis Their incidence was 70 %

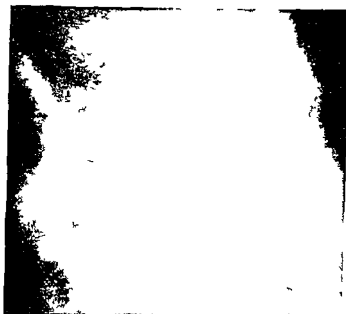


Fig. 4. Female 59 yrs with RA for 9 yrs. Bone ankylosis of the MSA. Thick layer of calcium substitutes the former joint space. On the left an erosion extending to both joint surfaces with demarcation wall.

or of the same order as previously reported in ankylosing spondylitis. The incidence in the control group was 27 %, which is identical with that observed by Dilsen et al. in non rheumatic subjects. Erosion was the most common change in the whole material studied and was present in 59 % of cases of rheumatoid arthritis and in 12 % of control subjects. Furthermore, a difference in degree was clearly evident. Erosions were accordingly fairly typical of rheumatoid arthritis, a common additional

TABLE I

Radiological Changes in the Manubrio-Sternal Articulation

	Erosions	Reactive sclerosis	Ankylosis	No changes	Total number of cases
Rheumatoid arthritis	51	4	7	5	6
Controls	1			[illegible]	95

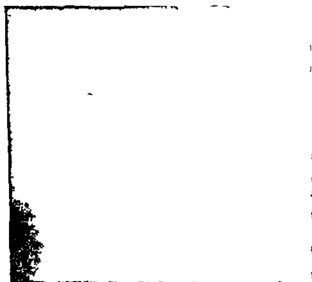


Fig 5 Female 31 yrs with advanced RA In the MSA osseous ankylosis The former joint space is poorly defined by calcifications

finding was the presence of also other changes more often than in the control group i e osteoporosis and marginal sclerosis Ankylosis of the MSA was seen in a total of 14 cases and in most of these it was of secondary or sclerotic type according to Ashley Primary fusion in which the site of the joint space could not be distinguished was seen in only three patients one of whom had rheumatoid arthritis

In many cases the erosions and osteoporosis were so considerable that they had to be regarded as strongly indicative of a rheumatoid process In fact in two patients the erosive changes in this articulation appeared before changes in other joints and thus were of importance for the early diagnosis of the disease (Figs 1 and 2) Moderate and slight changes however were the most common ones and in such cases the possibility to make diagnostic conclusions is slight This has been clearly demonstrated by Kormano (in this issue p 47) whose microradiographical and histological studies showed that radiological changes of the same appearance may arise on the basis of a rheumatoid process or a degenerative change of the MSA or of a combination of the two

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A MICRORADIOGRAPHIC AND HISTOLOGICAL STUDY OF THE MANUBRIO STERNAL JOINT IN RHEUMATOID ARTHRITIS

By

MARTTI KORMANO

Summary The manubrio-sternal joints (MSJs) of 10 male and 10 female non rheumatoid subjects and of 11 subjects with RA were studied Microradiography often revealed herniation of the intra articular fibrocartilaginous disc into the cancellous bone and osteo arthritic changes In RA erosive destruction of the articular surfaces was seen in six cases and in one subject the joint was ankylotic Histologically the MSJ presented degenerative phenomena but no inflammatory changes in old non rheumatic subjects Active inflammation was visible in four joints from rheumatoid subjects and in another four fibrous replacement suggestive of earlier inflammation was seen The joints from non rheumatoid subjects presented a histological joint cavity in nine cases, with or without synovial like cells lining the cavity A similar cavity was present in only one of the joints taken from rheumatoid subjects and there were inflammatory changes in the synovial tissue in this joint It is suggested that involvement of the MSJ in RA may be related to the occurrence of synovia in MSJ It is also stated that similar roentgenological changes in MSJ may arise due to an inflammatory as well as due to a degenerative process

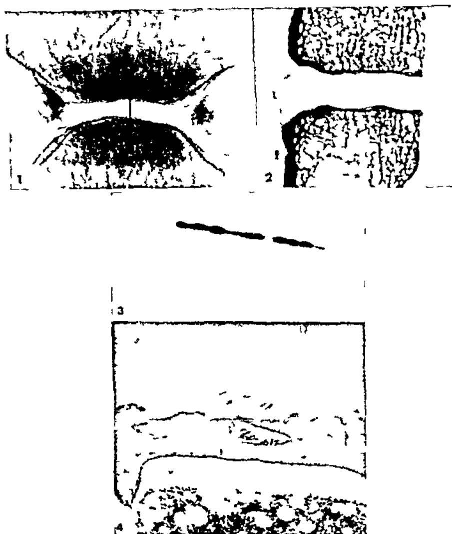
The manubrio sternal joint (MSJ) is an amphiarthrodial joint with hyaline cartilage covering the articulating ends of the manubrium and corpus sterni and a fibrocartilage between them Occasionally the joint becomes ossified as a result of either an early primary synostosis or a secondary sclerotic synostosis which occurs during late adult life

(2) In more than one third of cases there is a joint cavity (7) This is said to be *more frequent in women and in elderly persons* (6) The similarity with the pubic symphysis has led some authors to call the joint the symphysis sterni (12) On the other hand Stibbe (17) has claimed that inclusion of symphyses with synchodroses is illogical and misleading because symphyses have much in common with synovial joints The dual character of the MSJ has consequently led some authors to classify it as a diarthro-amphiarthrosis (18)

In ankylosing spondylitis numerous authors have reported changes in the MSJ (1 3 4 13 14 16) This disease primarily affects the true synovial joints of the trunk while the amphiarthrodial joints be tween the vertebral bodies not being synovial are not primarily affected except for calcification of the spinal ligaments Another joint often involved in this disease is the symphysis pubis a joint fundamentally similar to the MSJ (4 5)

In general rheumatoid arthritis (RA) is another disease attacking synovial joints However several authors have observed that changes in the MSJ can also occasionally be seen in RA (4 8 9 14) In most cases the changes described have been ankylosis slight irregularity of the bone ends and reduced height of the fibrocartilage Histological evidence of the nature of the lesions is sparse In ankylosing spondylitis Romanus and Yden (15) found active inflammation in one of the two cases studied In four cases showing radiographic erosions of the MSJ in ankylosing spondylitis Savill (14) found no inflammatory reaction but only fibrous replacement and in one case of RA he could not detect any histological changes at all

Recently Laitinen et al (10) observed that erosions and other radiographic changes in the MSJ can be seen in a high percentage of patients with RA However somewhat similar changes were also observed in some non rheumatoid patients Long ago Passler (11) reported that irregularity of the articular surfaces of the MSJ is relatively frequent in normal human subjects especially in old age and that sometimes herniation of the *articular cartilage into the bone occurs as in the vertebral* bodies He also mentioned that arthritic changes may occur in the MSJ To ascertain the true nature of the deformities visible radiographically in the MSJ of both rheumatoid and non rheumatoid patients a microradiographic and histological study was performed It was hoped that a knowledge of any microscopic processes which might be associated with the changes observed in this joint would help to elucidate the nature of this joint



Figs 1—4 Illustrations of the normal MSJ of a 55 year old male (1) Gross radiograph (2) Microradiograph x 3 (3) Histology of the joint cavity with oval cells lining the joint cavity x 800 (4) Histology of the well preserved hyaline cartilage on the sternal margin x 00

MATERIAL AND METHODS

The series comprises 31 autopsied cases from the Medical Depart of Kivela Hospital Helsinki Of these 10 male and 10 female



Figs 5—6 Non-rheumatic 81 year old female (5) Microradiograph of the cancellous bone with numerous indentations in the articular surfaces x 4 (6) Histology of an indentation a local breakage of the thin end plate containing a heavily vascularized fibrous nodule in direct contact with the bone marrow No inflammatory changes x 80

Figs 7—9 Non-rheumatic 63 year-old female (7) In gross radiograph the joint space is irregular and shows a bridge-like dorsal osteophyte (8) Sagittal microradiograph shows cortical degeneration, irregular joint surfaces and areas suggestive of erosions x 3 (9) Histology reveals herniation of the degenerate fibrocartilage end plate through the bony joint surface The fibrous nodule shows no signs of inflammation x 80

TABLE I

Microradiographic Findings in Manubrio Sternal Joints of Non Rheumatoid and Rheumatoid Subjects

	Joint cavity	Osteo phytes	Scle rosis	Inden tations	Ero sions	Osteo porosis	Anky losis
Non rheum subjects (20)	9	10	6	14	3	11	—
Subjects with RA (11)	1	8	7	7	6	5	1

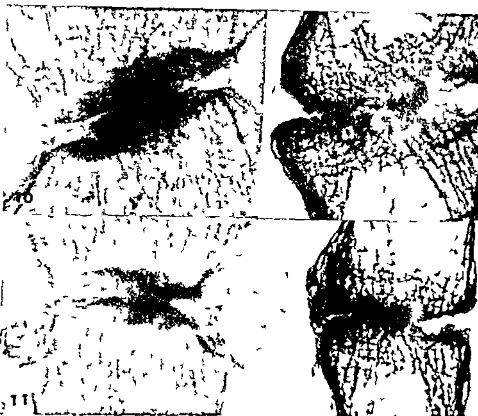
showed no signs of any rheumatic disease and served as control material Eleven MSJ samples were from cases with definite or classical RA (2 males and 9 females) The age distribution of the control series was 38—93 years (mean 69 years) and that of the RA series was 59—78 years (mean 68 years) The excised MSJs including a small part of the manubrium and corpus sterni were radiographed and fixed in formalin Sagittal sections 1 5—3 0 mm thick were prepared with a thin bladed saw These sections were microradiographed in contact with a fine grain x ray film (Microtex Kodak) *Further the sections were decalcified* and embedded in paraffin wax and 10 μ sections were stained with hematoxylin eosin and Weigert van Gieson techniques for histological study

RESULTS

MSJ of Non Rheumatoid Subjects

Gross radiography The joints presented various irregularities and senile degenerative changes in both male and female subjects Obliquity or asymmetry (Fig 10) of the joint space was seen in 6 cases Sclerosis and thickening of bone margins were common (in 11 cases) In older subjects there were often osteophytes (Fig 7) up to a sclerotic bridge between the manubrium and corpus Compared with a normal one (Fig 1) the joint surface was often irregular and indistinct but no erosions could be identified in gross radiographs

Microradiography Radiography of thin sagittal sections revealed many new features not visible in the ordinary antero-posterior radiograph Especially the narrowing of the joint space *and changes in the joint* surfaces were easily identifiable (Table I) A joint cavity was evident in nine subjects



Figs 10—13 Radiographs of different MSJs In each figure the gross radiograph from the whole MSJ is on the left and on the right there is a sagittal microradiograph of the same joint (10) Non rheumatic 90 year old male An asymmetric joint with a bony bridge Microradiography as well as histology shows degeneration of cartilage and bone but no signs of inflammation (11) Female 0 years RA for 14 years The gross radiographic and microradiographic appearance is very similar to that in fig 10 but histologically evident rheumatoid inflammation (12) Female *RA for 10 years Bony fusion was roentgenologically stated already 7 years before death No inflammatory changes in histology but only a hyaline remnant of the joint space* (13) Female 67 years, RA for 10 years The radiographs show destruction of the *joint with erosions and lipping Histologically there was fibrous ankylosis with rem*nants of the fibrocartilage No inflammatory reaction

Microradiography was especially suitable for the study of the bone margins Indentations of the articulating surfaces (Fig 5) were common However they had a smooth sometimes sclerotic contour and only three of them presented an eroded surface The thin sections were further



found to reveal the existence of osteoporosis very well as a rarefaction of the sternal cancellous bone

Histology The histological sections revealed no marked degenerative changes in subjects below the age of 65 years The articular surface was smooth and the hyaline cartilage on it was in good condition Subchondrally there was a thin layer of bone immediately below which was hematopoietic tissue The fibrocartilage between the two layers of hyaline cartilage was connected to the dorsal and ventral surfaces of the sternum by ligamentous structures In those cases where a joint cavity was found it was often a cell free hole within the cartilage but occasionally it contained hyaline non cellular material A few cases were found in which the joint cavity was partially lined with small oval cells (Fig 3) resembling synovial cells The degenerative phenomena observed in senile MSJs were first seen in the hyaline cartilage This layer was narrow and degenerate in most of the joints from subjects over 70 years

old The height of the fibrocartilage was often fairly well preserved even in cases with degeneration of the hyaline cartilage However patchy areas of degeneration and narrowing were also seen in this part of the joint in cases of marked senile degeneration In the degenerate joints blood vessels were seen to invade the joint from the dorsal ligament

Special attention was paid to indentations and erosions In most cases the thin end plate of the younger subjects was seen to form a simple indentation which was either filled with hyaline cartilage or occupied by a small fibrous nodule between the cartilage and the bone (Fig 6) Osteoclasts were often seen in these areas but no signs of true active inflammation could be detected in spite of the radiographic erosion visible in three cases Some of the indentations observed in microradiography proved to be true herniations of the fibrous cartilage into the bone in connection with rupture of the bony end plate and degeneration of the hyaline cartilage (Fig 9) Blood vessels and small fibrous nodules were formed between the herniated cartilage and the bone marrow No signs of active inflammation were visible

MSJ of Subjects with RA

Gross radiography In the eleven samples even gross radiography often revealed marked pathological changes In one 70 year old female there was complete ankylosis of the MSJ A sclerotic bridge between the two bones was seen in four of the other ten joints There was erosive destruction of the bone ends in both male sterna and in four female joints Sclerotic changes were common in the bone peripheral to the erosions

Microradiography The frequencies of the various changes are presented in table I Besides definite erosions in six cases (Figs 13 15 19) there were various pocket like indentations in the joint surfaces which had a relatively smooth sclerotic wall In three specimens no evidence of destructive or erosive joint changes was visible

Histology In the ankylotic joint a small hyaline mass was seen at the site of the joint space but there were no signs of inflammation Among the other 10 joints two showed no changes except those due to age (70 and 75 years) In four joints taken from subjects with a long history of RA there were no signs of active inflammation but the hyaline cartilage was degenerated and nodules of fibrous tissue were seen between the cartilage and the bone The end plates were thin and grooved



Figs 14—17 Male 67 years RA for 7 years (14) Gross radiograph shows narrowing of the joint space surface erosion and marginal bone sclerosis (15) Microradiograph reveals deep erosions in both articulating surfaces surrounded by sclerotic trabeculae x 4 (16) Histology from the area a in fig 15 In the joint cavity a hyaline and edematous piece of synovial tissue with mononuclear and polymorphonuclear cell infiltration In the subchondral area there is granulation tissue and inflammatory cells x 80 (17) Histology from the area b in fig 15 The whole hyaline cartilage and a great part of the subchondral bone has been replaced by granulation tissue which shows mononuclear cell infiltration and giant cells x 80

The fibrocartilage also showed degeneration The extensive fibrous replacement of the joint structures and the eroded bone gave the impression of earlier inflammation Four joints presented signs of chronic in



Figs 18—21 Female 78 years RA with rapid progression for years (18) Gross radiograph shows osteoporosis and narrowing of the joint space but no evident erosion (19) The microradiograph shows erosions in the sternal margin degeneration of the cartilage and advanced osteoporosis x 4 (20) Histologically an inflammatory nodule and destruction of cartilage and bone can be seen x 80 (21) High power view shows a giant cell and numerous mononuclear and polymorphonuclear cells within the rheumatoid nodule x 800

flammation with infiltration of the subchondral bone and hyaline cartilage by granulation tissue giant cells and mononuclear and polymorphonuclear cells (Figs 17 20 21) There was clear cut destruction of bone and cartilage (Figs 17 20) Only in one case was there a microscopically

detectable joint cavity In that joint partially hyaline edematous synovial tissue projected into the cavity from the dorsal ligament The synovial tissue was invaded by mononuclear cells (Fig 16) In two cases one with active inflammation and another with fibrous replacement there was a hyline layer at the site of the joint cavity

Correlation between Radiographic Changes in MSJ and the Histological Picture

In cases with erosive changes in the radiographs taken from RA patients there may be true inflammation in the MSJ or changes suggestive of earlier inflammation Apparently erosive changes as studied radiograph ically may contain pure degenerative or fibrotic tissue and herniated nodules of fibrous carilage On the other hand senile patients often have marked osteoarthritic changes around and within the joint and these changes may mask the arthritic process Histologically typical arthritic changes may be detectable in the MSJ of RA patients even in a joint that in gross radiography appears unaffected thin slice microradiography reveals these small erosions (Figs 18 19)

DISCUSSION

Occasional involvement of the MSJ in RA has been detected roentgenologically by numerous authors and the larger series of both Dilsen et al (4) and Laitinen et al (10) have shown that this finding is relatively common However the true nature of these changes has been disregarded in the previous literature except for a single report on ankylosing spondylitis (13) The present study has clearly demonstra ted that when clinically active RA is present in other joints true inflammation may also exist in the MSJ On the other hand if the history has been very long there may be only fibrous replacement masked by secondary osteoarthritic changes or even absence of changes suggestive of inflammation The changes observed in the chest x ray must there fore be carefully evaluated since even the microradiographic patterns of a rheumatoid and an osteoarthritic MSJ may be very similar (Figs 10 11)

The existence of a joint cavity in numerous non rheumatoid MSJs but only in one of those with RA raises the interesting question of the dual character of the MSJ and its relationship to the occurrence of rheumatoid changes in the joint A joint cavity is said to exist in slightly mo e than

one third of MSJs (7) The frequent occurrence of a joint cavity in the small control material presented here (9/20) can be considered to be of the same order although the observation has no statistical significance In only one MSJ from a RA patient was a histological joint cavity seen and it presented signs of synovitis On the other hand in the two joints from RA patients which showed no microscopic inflammatory or fibrotic change there was no joint cavity either It can be speculated that the MSJ may be involved in RA in those cases where a synovial cavity and/or some synovial cells are present but that the inflammation leads to the disappearance of the joint cavity while the inflammatory process continues in the hyaline cartilage and the subchondral bone The occurrence of marked changes in 53 % of MSJs in RA in the series of Dilsen et al (4) fits with this hypothesis In the series of Laitinen et al (10) gross erosive involvement was seen in as many as 70 % Unfortunately no information on the frequency of microscopic synovial rudiments in the MSJ is available

The destructive lesions in the MSJ were confined to the horizontal articulating surfaces of the joint and no juxta articular changes were detected The pattern of destruction therefore differs from that of a typical diarthrodial joint like the joints of the hand in which juxta articular erosions often appear (15) This might also be related to the limited distribution of both the synovial cavity and the hyaline articular cartilage in the MSJ

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STUDIES ON THE SICCA SYNDROME IN PATIENTS WITH RHEUMATOID ARTHRITIS

By

SUNE ERICSON and ERIC SUNDMARK

Summary Secretion from the parotid and the lacrimal glands has been studied in two similar groups of 48 subjects one consisting of RA patients the other a control group Sialometry and sialography has been performed as regards the parotid glands the secretion of the lacrimal glands has been measured with Schirmer s test I and the occurrence of conjunctival and corneal epithelial defects stainable with Rose Bengal has been studied

It has been shown that lacrimal and parotid secretion was significantly reduced in the RA group as compared with the control group that epithelial defects typical for KCS occurred significantly more often in the RA group that parenchymal changes sialodochiectasias existed in the RA group but that sialodochiectasias were completely absent in the control group that there was no significant co-variation in the RA group between lacrimal and salivary secretion or lacrimal secretion and sialodochiectasias that criteria for Sjögren s syndrome were fulfilled by 15 subjects in the RA group The results of the study indicate that Sjögren s syndrome is to be regarded as part of the RA complex.

It is widely held that rheumatoid arthritis (RA) is a generalized disease in which articular symptoms are the most prominent feature The

association of keratoconjunctivitis sicca (KCS) and articular changes was first noted by Houwer (1) and confirmed by Isikowitz (2) but since the publication of the detailed report on 19 cases by Sjogren (3) and his subsequent papers (4—7) with a description of in all 80 cases in which oral parotid and skin changes were also observed the syndrome has become known as Sjogren s syndrome (SS) which means the triad arthritis KCS and xerostomia or at least two of these three features

The literature on SS reveals that the condition still presents many unsolved problems In 1933 Sjogren declared that the syndrome was a well defined disease sui generis and that chronic polyarthritis was a part of SS and furthermore the commonest component (3) of the syndrome In subsequent works he has confirmed this view (8—9) Weber Ellman et al like Coverdale (10—12) state on the other hand that the mani festations of SS were merely the local symptom of a generalized disease or disturbance of unknown etiology In support of this thesis Holm (13) states that ocular changes (KCS) are rather common among rheumatic subjects (14 2 %) even if subjective symptoms are absent and that RA has etiological significance to KCS Congruent conclusions have been communicated by Reader et al (14) Lackington et al (15) Gaulhofer (16) Thompson & Eadie (17) and Bremova et al (18) who have reported frequencies of KCS in cases of RA varying between 9 and 58 per cent The connection between RA and SS has also been stressed by Bunim and Bunim et al (19 20) and Bloch et al (21) who express that the presence of a rheumatoid factor should be reckoned as a fourth main symptom in the sicca syndrome On account of serolog ical similarities with the auto immune diseases they consider that SS should be accounted as belonging to the collagenoses as are RA and LED but at the same time be regarded as a disease sui generis A contrary view on the role of RA as an etiological factor and part of SS has been advanced by Henderson (22) and Stoltze et al (23) who have examined 121 and 248 individuals with KCS respectively referred for ophthalmological examination They regard RA as simply an incidental finding without etiological importance for the sicca syndrome The same opinion has been put forward by Sachsenweger (24) Increased lacrimal secretion in RA cases as compared with that in persons healthy in this respect has been reported by Blatz (25) and Bucci & Stirpe (26)

As regards the third sicca component xerostomia the connection between this symptom (hyposialia) and RA has been investigated by Gunther (27) Lenoch et al (28) and Ericson (29) Günther and

Lenoch et al found a tendency to lower salivary gland secretion In a comparison between cases with RA and comparable controls healthy in this respect Ericson found significantly lower parotid secretion among the rheumatic subjects It was also possible to register a qualitative difference in xerostomia (30) The observation that hyposialia is more frequent in individuals with RA than in healthy subjects is in agreement with morphological findings reported by Waterhouse (31) Waterhouse & Doniach (32) Ericson (29 33), and Whaley et al (34) who have shown that pathological states of an inflammatory nature (lymphocytic or myoepithelial adenitis) are commoner in lacrimal and salivary glands in individuals with RA than is the case in healthy subjects The literature on SS shows that there are still representatives for diverging views concerning the etiology of the syndrome especially its relation to RA As Sjogren has already pointed out one contributory cause of diametrically opposed conceptions of the syndrome its components frequency and etiology may be a varying and vague definition of the RA concept The lack of uniformity in the classification and assessment in this connection is striking even in works of more recent date What should probably also be considered a relevant factor is that no all round investigation of the most important component parts of the syndrome with simultaneous comparison with an adequate control material has yet been carried out

In the present paper an account is given of a clinical examination of individuals with and without RA with respect to the occurrence of KCS and xerostomia The study is intended to throw light upon the co-variation between the three main components of the sicca syndrome

MATERIAL

The material in this investigation consists of two populations a group with rheumatoid arthritis (RA) and a control group (C)

Rheumatoid Arthritis Group

The material consists of in patients at the Rheumatological Subdivision of Internal Medicine at the Umea Hospital All the patients who at the time for the investigation were hospitalized and who according to the criteria of the American Rheumatism Association (ARA) had been diagnosed as cases of RA (classical definite or probable (35)) were requested to take part in the investigation Thus no patients with other

collagenous diseases or only ankylosing spondylitis are included in the group

Of 53 individuals in all it has been possible to use 48 32 women and 16 men while five have been excluded for various reasons The age among the women has varied between 26 and 62 years the average age being 48 5 years while among the men the age has varied between 26 and 64 years with an average age of 48 8 years The distribution of the sexes is in agreement with what is normally found among rheumatic subjects (36)

The classification of the material with reference to the diagnoses clas sical definite or probable RA was made on the basis of the criteria established by ARA Of ARA s eleven criteria 1—9 were recorded in all cases while this was not possible for 9—11 except in a few cases The diagnostic criteria therefore agree in the main with those used by Kellgren et al (37) for population studies A classification with respect to the progression of the disease has been made on the basis of the para meters defined by Steinbrocker s Committee (38) The classification in terms of anatomical stage has been based on the radiographic examina tion of the joints (39) and the most exposed joints have been qualifying for the stage The radiographic investigation has been complemented with respect to fibrous ankylosis contracture of tendons and ulnar deviation — states which it may be difficult or impossible to diagnose with certainty on the radiograph

All the individuals were considered to be in a clinically active phase assessed on the basis of articular swelling morning stiffness tenderness and pain in connection with movement Symmetrically localized swelling of the metacarpophalangeal — midphalangeal joints was recorded in almost all the cases Swelling in wrist and/or ankle was also recorded in the majority of cases

Control Groups

As earlier investigations have indicated that age and sex factors may affect the secretory volume for salivary and lacrimal glands (25 40—43) the control material has been chosen from the clientele of the School of Dentistry in Umea in the way described below

Taking the age and sex distribution of the RA group as the point of departure a twin has been selected at random by taking as from Janu ary 1965 from the registration list of the School of Dentistry s open reception the first individual domiciled in Umea who in respect of sex

TABLE I

Diagnostic Data for 48 Individuals with Rheumatoid Arthritis

Diagnosis (ARA)	Sex	N	Age (years)		Duration of the disease (years)		Rheumatoid progression stage (Steinbrocker et al 1949)			ESR		Pathological titers				LL cells	WR
			$\bar{x}$	range	$\bar{x}$	range	1+2	3	4	$\bar{x}$	range	SSCAT $\geq$ 1/80	AFT $\geq$ 1/40	AST > 00	ASTA > 5		
Classical RA	F	[illegible]	51.0	40—61	11.1	1—[illegible]	3	10	7	49	14—95	16	19	2	2	1***	0
	M	[illegible]	49.6	26—61	9.8	0.5—[illegible]	[illegible]	4	3	50	[illegible]1—106	7	9	0	1	0	0
Definite RA	F	10	43.7	26—[illegible]	[illegible]	0.5—17	[illegible]	1	3	35	13—52	[illegible]	3	1	0	0	0
	M	7	47.[illegible]	33—63	[illegible]	0.5—7	7	0	0	35	[illegible]—74	4	6	2	3	0	0
Probable RA	F		46.5	31—62	1[illegible]	1—1.5		0	0	34	[illegible]1—47	0	0	0	1	0	0
	M	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

* Waaler Rose test

** Acrylic flocculation test ad modum Winblad

** 1 or 5 tests positive

TABLE II

Diagnostic Data for 48 Individuals in the Control Series

Sex	No	ESR $\bar{x}$	ESR range	Pathological titers SSCAT ≥1/80	AFT* >1/40	AST > 00	ASTA > 5	WR	Erosive changes in x-rays of the joints of the hands
F	32	11	[illegible]—33	0	1	7	1	0	0
M	16	8	3—[illegible]1	0	0	5	2	0	0

* In dilution 1/10 1 aggl 1/20 2 aggl 1/40 2 aggl
** In dilution 1/20 2 aggl

and age ±2 years has had a counterpart in the RA series which was primarily set up in alphabetical order. If the random selected control individual had a diagnosed RA then the person in question has been excluded and replaced by the next in order who met the requirements mentioned. There have been no other grounds for exclusion. According to the ARA criteria there is no individual in this group who fulfills the conditions for RA. Among a total of 52 control cases asked for examination there was a non response of four. The control group thus comprises 48 persons with the same sex and age distribution as in the RA group.

None of the individuals in the C group was acutely ill at the time of the examination or suffering from a disease which according to earlier experience has proved to give rise to disturbances in the lacrimal or salivary secretion.

Diagnostic Data

The RA group and the C group have been included as part material earlier in a study by one of the authors (29) and constitute the 48 cases last examined in that investigation. As in that study a detailed account is given of the results of the general medical and rheumatological examinations, methods and studies of systematic errors, the reader is referred to it for a detailed presentation. Table I gives a brief account of the composition of the RA group with respect to the duration of the disease, the severity and activity as reflected in the laboratory values. The corresponding tabulation for the C group is given in Table II.

METHOD

All individuals have been examined in the following respects

A Sialometric and sialographic examination of the left and right parotid glands

B Ophthalmological examination with special reference to the sicca syndrome of the left and right eyes

Data have been collected according to a definite pattern and data processed with current statistical methods (29)

Ad A

The following examinations have been performed

History with particular emphasis upon the sicca syndrome and current or earlier pathological conditions in the regions of the face or neck

Clinical examination with recording of extra oral and intra oral symptoms

Recording of the parotid flow rate with the method described by Diamant & Wiberg (44) and modified by Ericson (29)

Sialometry of both the salivary glands has been carried out simultaneously during rest and during continuous stimulation with 1 % and 6 % citric acid respectively The saliva has been collected with a suction cup placed over papilla salivaria in cavum oris Via a water filled system the collecting cup has been connected with a drip chamber in which the drops have fallen past a photo electric cell The impulses from the latter were transferred to a mingograph (Elema Schonander) with which every impulse (drop) has been automatically reproduced graphically Every drop has consisted of 0 06 g saliva (29)

Radiographic examination of the left and right parotid glands has been performed with sialography (29)

For a detailed account of the method and of methodological studies concerning the sialometrical and sialographic methods the reader is referred to an earlier work (29) The errors of the methods are small throughout

Ad B

Recording of history with special attention paid to KCS *After determination of* visual acuity and refraction focal illumination, ophthalmoscopy and scrutiny of cornea and conjunctiva by slit lamp After examination in unstained state application with glass rod with-

previous surface anesthesia of a small amount of 1 % Rose Bengal solution on the insides of lower eyelids Immediately after spreading of the staining substance by voluntary blinking renewed examination by the slit lamp with respect to stainable epithelial defects on cornea and conjunctiva Occurrence of such defects was recorded with the following gradation

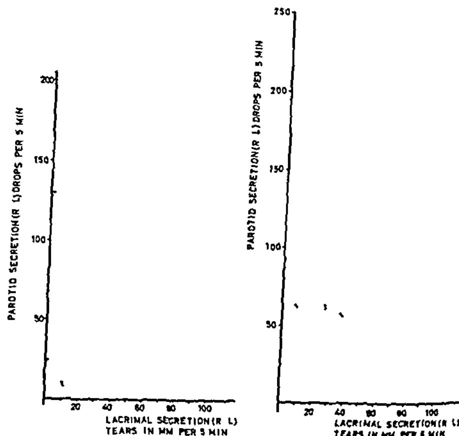
0 No defects
I A few small defects on conjunctiva and/or cornea
II Several defects
III Abundant occurence of defects

After an interval of 15 minutes Schirmer s blotting paper test I was performed The same kind of litmus paper (precision red litmus pa per) 6 x 48 mm was used throughout The folded in part of the strip measured 3 mm and the strips were placed over the ciliary border between the middle and the outer third of the lower eyelids The measurement in millimeters of the moistened part of the strips outside the fold was performed after 5 min When the whole of this part was moistened in a shorter time than 5 min the value of 45 mm was recorded This occurred in three RA cases (3 eyes) and four C cases (6 eyes)

Some idea of the precision of Schirmer s test I may be obtained by calculating the correlation coefficient between the right and left eyes in the control series The coefficient $r = 0.74$ indicates in the light of the fact that a certain biological variation between the right and left eyes may be expected, that the systematic error is moderate as compared with the variation in secretion between individuals (Table III) The mean for the left and right eyes and the standard error of the mean are of the same order of magnitude which is also evidence of acceptable precision

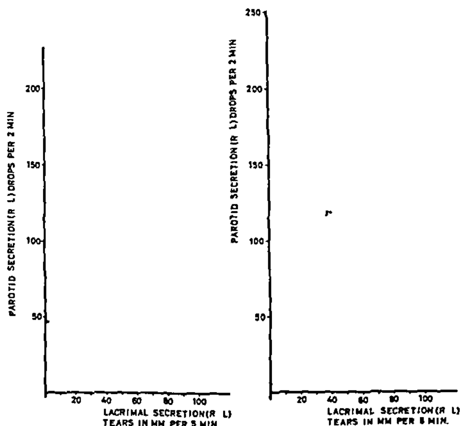
RESULTS

The mean values ($\bar{x}$) the standard error of the mean value ($SE_{\bar{x}}$) and the lowest and highest values (range in lacrimal and salivary secretion) for both the RA and C groups are given in tables III—VI showing that the RA group s mean values for lacrimal and salivary secretion are in all cases lower than the corresponding values for the C group Within the groups however the scattering is considerable especially among the men



Diagrams 1 and 2 Co-variation between lacrimal secretion (Schirmer's test 1) *and parotid secretion stimulated with 1 % citric acid in individuals with RA (diagr 1)* and in healthy controls (diagr 2) Every *point corresponds to an individual*

The statistical processing as regards eventual differences in lacrimal and salivary secretion between the RA and C groups was carried out by formation of quotients on the twin pairs formed in the account of the material The twin pairs consist of an RA individual and a randomly chosen C individual *of the same sex and age* according to the criteria earlier referred to The calculations are based on a difference in *lacrimal and salivary* secretion, respectively between the 48 pairs The difference between the test values for RA and C cases has been calculated within the pair after which this difference has been divided by the sum of the same test values The quotient has been designated $\frac{R-C}{R+C}$ where R stands for the value for the individual with RA and C for the corresponding value for the control Theoretically such quotients may vary from —1 to +1 and should on an average be equal to 0 if there is no difference between RA cases and C cases The results are given in table VII



Diagrams 3 and 4 Co variation between lacrimal secretion (Schirmer s test I) and parotid secretion stimulated with 6 % citric acid in individuals with RA (diagr 3) and in healthy controls (diagr 4) Every point corresponds to an individual

From table VII it may be seen that the secretions for the RA group are on an average lower than those for the C group As regards the secretion of the salivary glands the difference is strongly significant ($P < 0.001$) as regards the Schirmer s test not so strongly certainly yet still significant ($P < 0.01$) (Analysis of significance performed with t test) From table VII it is further evident that negative differences are commoner than positive differences but also that there are rather a large number of positive differences

The question as to whether the positive (and negative) differences in the Schirmer values are accompanied by positive (and negative) differences in the values for parotid secretion has been analysed Table

TABLE III

Lacrimal Secretion according to the Schirmer's Test I

Figures refer to 48 subjects with RA and to 48 controls (Mean values standard errors of the mean and lowest and highest values for right and left eyes in mm)

Sex	RA			C	
	No	$\bar{x} \pm SE_{\bar{x}}$	range	$\bar{x} \pm SE_{\bar{x}}$	range
F	32	30 2±4 2	2—90	34 4±[illegible] 9	8—90
M	16	22 5±3 9	1—45	36 7±5 6	9—90

TABLE IV

Parotid Secretion at Relative Rest in Number of Drops/5 Min

Figures refer to 48 subjects with RA and to 48 controls Left and right glands (Mean values standard errors of the mean and range)

Sex	RA			C	
	No	$\bar{x} \pm SE_{\bar{x}}$	range	$\bar{x} \pm SE_{\bar{x}}$	range
F	32	4 7±1 0	0—26	4 7±0 9	0—[illegible]1
M	16	10 3±3 3	0—45	9 4±1 7	0—[illegible]

TABLE V

Parotid Secretion Stimulated with 1 % Citric Acid in Number of Drops/5 Min

Figures refer to 48 subjects with RA and to 48 controls Left and right glands (Mean values standard errors of the mean and range)

Sex	RA			C	
	No	$\bar{x} \pm SE_{\bar{x}}$	range	$\bar{x} \pm SE_{\bar{x}}$	range
F	32	58 8±4 7	3—153	94 [illegible]± 5 [illegible]	31—158
M	16	72 4±2 7	17—200	107 [illegible]±17 0	23—[illegible]

TABLE VI

Parotid Secretion Stimulated with 6 % Citric Acid in Number of Drops/5 Min

Figures refer to 48 subjects with RA and to 48 controls Left and right glands (Mean values standard errors of the mean and range)

Sex	RA			C	
	No	$\bar{x} \pm SE_{\bar{x}}$	range	$\bar{x} \pm SE_{\bar{x}}$	range
F	32	76 7± 7 5	0—194	106 [illegible]± 5 8	60—[illegible]
M	16	91 0±14 1	28—218	1[illegible]8±13 0	31—[illegible]

TABLE VII

The 48 Pairs of the Material Distributed with Reference to the Quotient $\frac{R-C}{R+C}$ for Schirmer's Test I and for Secretion Test of Parotid Gland after Stimulation with 1 % and 6 % Citric Acid

R indicates subject suffering from RA, C a control subject (Mean values standard deviation and standard errors of the mean)

$\frac{R-C}{R+C}$	Schirmer's test I Number of pairs	Parotid secretion: 1 % citric acid Number of pairs	Parotid secretion: 6 % citric acid Number of pairs
—1 00 — —0 80	2	5	2
—0 80 — —0 60	7	5	7
—0 60 — —0 40	3	9	4
—0 40 — —0 20	9	7	8
—0 20 — 0	12	8	10
0 — +0 20	6	5	10
+0 20 — +0 40	4	6	6
+0 40 — +0 60	4	2	1
+0 60 — +0 80	1	1	—
+0 80 — +1 00	—	—	—
Total	48	48	48
$\bar{x}$	—0 15	—0 25	—0 19
SD	0 38	0 41	0 37
SE$\bar{x}$	0 055	0 059	0 053

VIII gives the correlation coefficients (r) between the different secretion values for the RA and C groups and for the RA group also the coefficients between secretion and the variable sialodochiectasias (see p 76) The table shows that the connections between the lacrimal and salivary secretion in the RA group are weak In the C group the corresponding connections are stronger and attain significance indicating a certain co variation (r = 0 40** and 0 38**) The difference for many pairs however both RA and C cases is positive for the Schirmer's test but negative for the salivary test That the weak connections are not a consequence of errors due to the method is evident from the fact that the correlation coefficients between the right and left sides are high for all three tests (Table X and Ericson (29)) A graphic account of the co variation between lacrimal secretion and citric acid stimulated parotid secretion is given in diagrams 1—4

TABLE VIII

Correlation Coefficient between Lacrimal Secretion and Parotid Secretion Stimulated with 1 % and 6 % Citric Acid (Right and Left Side) in 48 Individuals with RA and 48 Controls

Tests	RA						C				
	Resting secretion	1 % citric acid	6 % citric acid	Schirmer's test I	Rose Bengal	Sialodochiectasias	Resting secretion	1 % citric acid	6 % citric acid	Schirmer's test I	Rose Bengal
Resting secretion (R + L)		0.10**	0.34*	—0.03	—0.04	—0.08		0.36**	0.33*	0.18	—0.08
1 per cent citric acid stimulated secretion (R + L)			0.75***	0.[illegible]4	—0.16	—0.13			0.80***	0.40**	—0.05
6 per cent citric acid stimulated secretion (R + L)				0.15	—0.23	—0.12				0.38**	—0.09
Schirmer's test I (R + L)					—0.41**	—0.34*					—0.19
Stainability of the eyes with Rose Bengal (R + L)						0.36**					

(R + L) Right and left sides

P<0.05

P<0.01

P<0.001

TABLE IX

Stainability of Cornea

Studied with Rose Bengal in 48 subjects with RA and in 48 controls

Stainability with Rose Bengal	RA		C	
	Left	Right	Left	Right
0	37	37	45	40
I	2	4	3	7
II	8	6	0	1
III	1	1	0	0
Total	48	48	48	48

TABLE X

Correlation Coefficients between Right and Left Sides for Schirmer's Test I and Stainability with Rose Bengal in 48 RA Individuals and 48 Controls

Tests		RA				C			
		Schirmer's test I		Rose Bengal		Schirmer's test I		Rose Bengal	
		R	L	R	L	R	L	R	L
Schirmer's test I	R		0 90 **	−0 31*	−0 43**		0 74**	−0 1	−0 15
	L			−0 37*	−0 39 **			−0 16	−0 15
Stainability with Rose Bengal	R				0 88**				0 8*
	L								

* P<0 05
* P<0 01
* P<0 001

To sum up the RA group shows significantly lower secretory value than the C group with respect to all the tests described here However it has not been possible to deduce any convincing connection between the lacrimal and parotid flow rate for the RA series

As stated in the account of the method the stainability of conjunctiva and cornea with Rose Bengal is given in four degrees 0 I II and III The distribution of stainable epithelial defects according to this gradation for the right and left eyes is shown in table IX

It emerges from the table that stainable epithelial defects are commoner in RA cases than in C cases If we consider exclusively the

TABLE XI

Secretion Values (Right and Left Sides) according to Schirmer's Test I for Cases Stainable with Rose Bengal (II III) contra Non Stainable Cases

(Mean values standard errors of the mean and range)

Stainability with Rose Bengal	RA			C		
	No F + M	$\bar{x} \pm SE_{\bar{x}}$	range	No F + M	$\bar{x} \pm SE_{\bar{x}}$	range
Positive	9 + 1	12.2 ± 2.2	2—35	1 + 0	27.0	
Negative	21 + 14	32.5 ± 3.8	1—90	24 + 14	38.9 ± 3.2	8—90

TABLE XII

Cases Showing Either Stainability of Eyes with Rose Bengal (II III) or Sialodochiectasias in the Parotid Gland in the RA Group

Case	Sex	Schirmer's test I mm/5 min		Stainability with Rose Bengal		Sialodochiectasias		Subjective symptoms of dryness	
		L	R	L	R	Unilat	Bilat	Eyes	Mouth
1	F	5	6	++	+++	—	—	bilat	+++
2	F	18	17	++	++	—	—***	—	—
3	F	9	9	++	++	—	—	—	(+)
4	F	1	1	++	—	—	—	—	—
5	F	4	2	++	++	—	—	—	—
6	F	45	[illegible]	—	—	—	+	—	—
7	M	13	8	—	+	—	+	—	+++
8	M	15	5	+	++	—	+	—	—
9	F	5	3	++	++	—	+	—	—
10	F	4	7	++	+	—	+	bilat	+++
11	F	2	3	+++	+	—	+	bilat	++
12	F	4	2	++	++	—	+	—	—
13	F	40	45	—	—	—	+	—	—
14	F	6	7	+	—	+	—	—	—
15	F	25	27	—	—	+	—	—	—

* see text page 66

** slight + moderate ++ severe +++

*** marked atrophy

stainability of degrees II and III the differences between the RA and C groups are striking and significant ($P < 0.01$) These degrees of stainability existed in the RA group unilaterally and/or bilaterally in

10 cases (= 21 %) 9 women and 1 man only bilaterally in 6 women (= 15 %) and 0 men In the C group there was only one woman with stainability of degree II unilaterally

Among the RA cases 11 cases showed stainability of some degree of the left eye and 11 of the right eye As the stainability refers to only 13 individuals this implies that if the one eye is stained with Rose Bengal then this often applies also to the other eye (r = 0 88*** table X) which may be seen as the effect of some general factor probably related to the RA In the C group the corresponding connection is weak (r = 0 28*) For the Schirmer s test the agreement between right and left eyes is close in both groups but closer for the RA group (Table X)

In table XI is given an account of the lacrimal secretion for the ten cases who showed stainability of degrees II or III on either eye The mean for the lacrimal secretion of these individuals is considerably less than the mean for the non stainable cases in the RA group ($P < 0\,001$) The connection between KCS and stainability of degrees II or III is thus evident Table XII shows however that the secretion varies to an appreciable extent also within this delimited group The correlation coefficient between stainability (I—III) and the volume of lacrimal secretion is r = —0 41** for the entire RA group

Morphological changes in the parotid glands have been investigated with sialography Biopsies have moreover been taken from a number of glands with radiographic sialodochiectasias with the aim of analysing the nature of the change Ten individuals 8 women and 2 men (21 %) show sialodochiectasias eight (16 %) bilaterally (Table XII) Corresponding formations are entirely absent in the C group The difference is significant ($P < 0\,001$) It may be observed that only one individual has had clinical symptoms of parotitis in the form of swelling tenderness reddening or pain

Sialodochiectasias (Fig 1) have occurred in large numbers and on the basis of the histological examinations they may be regarded as sure signs of sialadenitis which is with a high degree of probability of myoepithelial type (29) Table XII comprising cases with either sialodochiectasias or stainability of at least degree II shows that sialodochiectasias and epithelial defects in cornea and conjunctiva appear with the same frequency in the RA group but that only in less than half of the cases do the changes appear simultaneously An analysis of the lacrimal and salivary secretion for these 15 cases shows a congruent pic

population Five cases reported dryness in the eyes and eight in the oral cavity (moderate or pronounced) Of these two and four respectively proved not to have any objective sicca symptoms in the form of stainability of cornea or conjunctiva with Rose Bengal low secretory values or sialodochiectasias in the parotid gland Conversely only four of 15 individuals in all with any of these symptoms reported dryness in the eyes and/or oral cavity (Table XII) The correlation between subjective and objective sicca symptoms is weak

DISCUSSION

Comparisons between quotients formed show significantly lower lacrimal and parotid secretion in rheumatoid subjects compared with the corresponding secretion in healthy controls The results agree in this respect with the investigations of Reader et al Lackington et al Gaulhofer Thompson & Eadie and Bremova et al (14—18) and differ from the results reported by Blatz and Bucci & Stirpe (25 26) However it is difficult to make comparisons with other studies as the methods of randomisation and the criteria for RA vary as between different investigators

The weak co-variation between lacrimal and parotid secretion either in the RA or in the C group (Table VIII) may for the RA group be seen as an incongruence in the light of the demonstrated connection between RA and reduced lacrimal and salivary secretion However this only means that the two organs are not always affected simultaneously or in an equally high degree by the disease The same observation has been made in cases of manifest SS (9 13 21—23 28)

The criterion for KCS has been stainability of cornea or conjunctiva with Rose Bengal On account of the normally great variation between individuals (Table III) and the consequent difficulty of establishing a limit value between a pathological and healthy state the Schirmer value has been considered as uncertain as a parameter for KCS However only stainability of at least degree II has been considered to qualify for the diagnosis KCS as isolated epithelial defects may easily arise accidentally in consequence of external irritation The relatively high frequency of cases of stainability of degree I in the C group together with the striking difference in frequency as between the right and left eyes in this group may perhaps be explained in this way The connection between

lacrimal dysfunction and stainability of degrees II and III is confirmed by a high correlation coefficient between the variables even if individual deviations exist Localization and extent of epithelial defects in those individuals who have had stainability of degrees II and III have been in agreement with what Sjögren (8) reports as typical for SS

According to Sjogren (3 9) the syndrome dacryo sialo adenopathia atroficans (SS) exists when at least two of the three components arthritis KCS and xerostomia exist simultaneously The two last mentioned components are considered to represent morphological changes in the lacrimal and salivary glands respectively (3 21 45) Against the background of a normally great variation in salivary secretion and a weak correlation between the secretory output of the parotid glands and the subjective sensation of dryness in the oral cavity together with the circumstance that the function of the salivary gland is affected by a number of factors a more objective sign of parenchymal change than the parameter xerostomia would be preferable in helping to decide whether the salivary glands were engaged in a case of suspected SS

In this RA group as well as in an equivalent larger group biopsies have shown unequivocally that the sialodochiectasias on the sialogram must be seen as a sign of parenchymal change In the rheumatic subjects the sialodochiectasias are constituted with a high degree of probability by a chronic sialadenitis of myoepithelial type (29) As both myoepithelial sialadenitis and marked stainability of the eyes with Rose Bengal are considered to be pathognomonic signs of SS (3 8 9 13 46—49) the number of cases with SS in the investigation can be established In the RA group there are 15 cases fulfilling the criteria for SS (31 %) table XII In agreement with earlier studies of SS (2 13 21 23 25 45) there is a strong dominance for women The results seem to indicate that SS is of common occurrence among rheumatic subjects but that the commonest form is a subclinical manifestation The results are supported by earlier mentioned morphological studies (autopsy) of lacrimal and salivary glands in individuals with RA by Waterhouse (31) Waterhouse & Doniach (32) and Whaley et al (34) which have shown that concealed forms of SS seem to be rather common among individuals with RA

A comparison shows that the 15 cases in the RA group which fulfil the criteria for SS in accordance with Sjögren s definition (3 9) do not differ significantly from the other 33 individuals in the group with respect to the classification duration anatomical stage or laborat[illegible]

values of the RA These circumstances together with the high frequency of cases of the syndrome among individuals with RA and the fact that corresponding changes are absent in the control group support the assumption that especially in a milder form the syndrome is to be regarded as a part of RA or very nearly related to that disease This hypothesis is not contradicted by the fact that the syndrome may appear without clinical RA (21 23) Thus studies by Olhagen (50) Bunim (19) Block et al (21) have shown that even in those cases in which clinical RA is absent there is often a positive titer for the rheumatoid factor and also in other respects a typical serology of RA Biopsies from individuals with SS without clinical RA have also shown the same cell picture as the SS cases with clinical RA i e a sialadenitis of the same type as has been discussed in this paper (21)

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MALIGNANT SYNOVIOMA

By

ODD KOGSTAD

Summary Malignant synoviomas may clinically resemble localized arthritis and it is the rheumatologist who may be in a position to make the important early diagnosis The course of the disease may be rapid or prolonged as illustrated by two case reports The clinical histological and roentgenological features are described and a review of the treatment and prognosis is given

Malignant tumors of synovial origin are usually called malignant synoviomas (synovialomas) or synovial sarcomas It is an uncommon tumor only 8—10 % of tumors originating from connective tissue and para articular tissue They may arise from synovial membranes tendon sheaths bursae and intermuscular septae or fasciae

The first report of this disease was made by Langenbeck in 1865 A number of cases have been recorded in the last 30—40 years (2) We have recently had difficulties with the diagnosis in two patients their records may therefore be of some interest

CASE REPORTS

Case 1 K D was a 52 year old male with no specific familial history of rheumatic diseases who had been in good health In May 1967 he was admitted to the rheumatological department with an affection

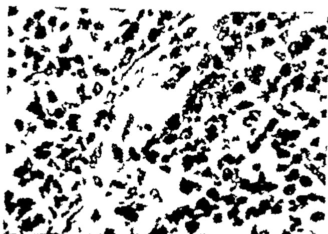


Fig 1

Case 1 Malignant synovioma of elbow The photomicrograph shows poorly differentiated tumor with marked variation in cells and nuclei and with areas with necrosis *The cells are partly polygonal partly spindle shaped The nuclei are prominent,* irregular partly vesicular and hyperchromatic In different parts of the tumor tissue are irregular glandlike spaces lined with the same atypical cells (160 1) (Det Norske Radiumhospital Laboratorium for Pathology)

of the right elbow starting with pain 5—6 months prior There was no history of trauma The elbow became gradually more painful a swelling was noted and movement became restricted No pathological findings were noted on roentgenological examination two months before admission ESR was then 48 mm/h There was little or no effect with indomethazin and phenylbutazone treatment and attempted physiotherapy gave more pain

On admission the range of movement was from 70—90 There w muscular atrophy about the elbow and a peri articular swelling wi some tenderness especially on the cubital side

No pathological findings were noted in other joints Laborato *examinations ESR 106 mm/h hemoglobin 88* %, Waaler Ros negative LE-cell test negative pirquet negative white-cell coun 5 800 electrophoresis of plasma proteins slightly raised alpha globulin serum transaminases and creatinine normal urine no pathologic findings

No abnormal findings were noted on x ray examination with results projections and planigraph including x rays of thorax and caput Th



Fig 2 *Fig* 3

Case 2 Roentgenogram of the right knee in 1958 (Fig 2) shows trace of calcium deposit behind the distal part of femur 7 years later (Fig 3) the picture shows irregular deposits of amorphous calcium in a soft tissue mass

patient was given phenylbutazone analgetics and antibiotics In two weeks he got gradually worse with general malaise slightly elevated temperature and glands were found in the axilla and the supra clavicular region on the right side He was transferred to the ortopedic dpt for explorative arthrotomy At operation a grayish tumor mass was found mostly located in the frontal part of the joint and apparently originating from the synovial membrane A wide excision of the tumor together with resection of capitulum radii and a synovectomy was done

In the post-operative course x rays of the chest showed pathological findings in the right lung In spite of antibiotic treatment his temperature rose his general condition deteriorated and he died 10 days after the operation Histologically we found the characteristic picture of a malignant synovioma (see fig 1)

Case 2 A 22 year old man in previously good health There was a history of trauma to his right knee in 1958 but no fracture was found on x ray examination (fig 2) In 1963 he had recurrence of pain in the knee without trauma He developed some stiffness and a little extension deficit was noted ESR and other laboratory tests were normal In 1965 repeated laboratory controls were normal Roentgenograms this time showed calcium deposits behind the distal part of the femur (see fig 3)



Fig 4

Case 2 Roentgenogram July 1967 shows osteolytic changes in the distal part of femur

This finding could be traced back to the first x rays taken in 1958 (fig 2) but at that time to a very slight degree Conventional physiotherapy gave no improvement The knee was immobilized for three weeks in an attempt to correct the extension deficit He had intermittent difficulties with his knee for the next two years In the spring and summer of 1967 his symptoms increased On admission to the orthopedic dpt in July of 1967 he had a range of movement of 20 to 75° and marked atrophy of the right thigh and calf ESR 26 mm/h white cell count 7 200 hemoglobin phosphatases Na K Ca P and chlorides showed normal values Roentgenograms now showed osteolytic changes in the distal part of femur (fig 4) and there were suspected metastases to the left lung (fig 5)

The patient was transferred to Det Norske Radiumhospital Oslo Additional examinations revealed a pathological angiogram with suspection of a malignant tumor in the knee region Roentgenograms showed metastases to both lungs A biopsy from the distal part of the



Fig 3

Case 2 Roentgenogram July 1967 shows metastases to the lungs

femur revealed histologically a malignant tumor of mesenchymal origin probably a malignant synovioma (see fig 6 and fig 7)

The patient received cobolt treatment with an average dose of 1 000 R In addition he was given Methotrexate 2 5 mg daily for 4 days 10 days intermission and a repeated dosage He was discharged and the same treatment with cytostatica was given ambulatory under close laboratory control He had a good remission after this treatment however in June 1968 he was readmitted to the ortopedic dpt He had been having increasing symptoms of general malaise dyspnea coughing and hemoptysis *Roentgenograms of the lungs had for some time been* showing increasing signs of metastases The patient died after a sudden rise in temperature

PATHOLOGY

Malignant synoviomas are usually located to the extremities and are reported more frequent in the lower than the upper extremities The



Fig 6 (75 1)

have also been found in the gluteal region on the neck chest abdominal wall and even in the orbita The variation of the size of tumor can be from one to above 20 cm in diameter Grossly the tumor is firm pink or gray and may have foci of calcification It is more firm if the cells are mostly of fibrous origin Softer tumors have more epitheloid cells with cystic elements containing a mucinous fluid with hyaluronic acid Because of the expansive growth with compression of surroundin tissues the tumor may appear encapsulated (pseudo capsula) Histologically the picture is often a caricature of normal synovial tissue

CLINICAL SYMPTOMS

These tumors occur most frequently in the twenties and thirties b have been reported from birth to 80 years of age In children they seem to be most frequent in the three first years of life (5) There seems to be a preponderance in men

Etiologically traumas have been recorded for shorter or longer periods before onset of symptoms with different frequency (12) The initial symptoms are dependent on the localization A superficial tumor localized to a tendon sheath or bursa will usually give earlier symptoms

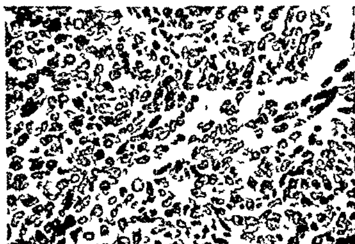


Fig 7

Case 2 Malignant synovioma of knee Biopsy of bone tissue from femur shows fibrous tissue with irregular spaces lined with atypical cells There are bundles of cells, partly polygonal, partly spindle shaped with round and ovoid nuclei Mitotic figures are abundant (160 ×)

and is discovered earlier than one in a deeper location Tumors located in the neighborhood of joints very often give similar symptoms as an arthritis and may often be referred to specialists in rheumatology The symptoms are pain peri articular swelling and restricted movement Pain is often the initial symptom and the misdiagnosis arthritis often persists too long The tumor also may be situated in the soft tissue at a shorter or longer distance from the joints where usually no synovial tissue is located In those cases tumor may arise from a metaplastic changed primitive tissue or embryonal synovioblast cells

DIAGNOSIS

An early diagnosis may be very difficult Malignant synoviomas should always be kept in mind in connection with tumors in the soft tissue of the hand and foot and in the regions of knees elbows shoulders etc

There are no specific roentgenographic characteristics for this tumor A soft tissue swelling can be noted para articularly In some cases the

tumor may contain clusters or mall foci of calcification (1 7) Tumor may invade neighboring bone giving rise to a periosteal reaction and osteolytic defects (cfr case 2) Arteriographic examinations may show greatly increased vascularity particularly in rapidly growing tumors The diagnosis can only be verified on histological examination Biopsy should not be undertaken unless there are adequate facilities for immediate histological study and eventually subsequent radical surgery It is stressed in previous reports (2 13) that partial exploration and incomplete resection should not be done

Soft tissue tumors can be divided into different groups but mainly the differentiation between the benign and the malignant is important. *Subsequently the exact diagnosis with histological verification of the* type of tumor must be done Of benign lesions a simple ganglion on the hand and foot or a bursa may be mistaken Xanthoma may have a similar localization

Myomas and chondromas usually have a harder consistence Lipomas and the special nodular synovitis also can be mistaken Among the malignant tumors fibro sarcomas lipo sarcomas and rhabdo myo-sarcomas can be differentiated by biopsy

PROGNOSIS AND TREATMENT

A benign appearance and a location with non specific symptoms often makes the diagnosis difficult and the prognosis poor The prognosis is also dependent on the grade of malignancy A long history is in the favor of a better prognosis when no signs of metastases are present The prognosis is better in children than adults (2 5) Berman (1) reviewed 314 cases reported in the older literature and found that only 8 % lived longer than five years after the onset of symptoms In Cade s series (2) of 46 patients who were free of distant metastases the five years disease free survival rate was 50 %

These figures must be accepted with the reservation that recurrences and metastases of these tumors may appear after a latent period of 10 years or more following surgery

There is general agreement that wide excision of the primary tumor should be done in cases without distal metastases The magnitude of resection depends upon the location extent of tumor and grade of malignancy Often an amputation at an appropriate level of an extremity

is required This is necessary when there are deep-seated lesions of hands feet or joints when there are local recurrences and when removal of extensive tumor masses would give functionally bad result

Malignant synoviomas have not proven sufficiently radiosensitive to be given irradiation alone However a number of patients have been reported cured by irradiation (13)

The prognosis is improved when post operative irradiation of the tumor area and the regional lymph nodes is given (1 2 13 14) Irradiation may also be useful in the palliation of patients with inoperable recurrences or metastatic visceral disease (1 2 14)

Cytostatica can give remissions and palliation Aminopterin azatioprin chlorambucil and actinomycin D (especially with children) have been mostly used (2)

A combined treatment with surgery irradiation and cytostatica may be used in palliation of some patients

COMMENTS

Our first patient represented the picture of a mono-arthritis Anti inflammatory treatment gave no response and mobilizing physiotherapy gave more symptoms He represented a fulminant course of the disease Invasion of regional lymph nodes and visceral metastases to the lungs developed in half a year The second patient had a trauma five years before the first symptoms and the course of the disease lasted more than five years It is possible that the tumor existed asymptomatically even prior to the trauma (cfr calcifications on roentgenograms from 1958) Only in the last two years of the disease were elevated ESR s and roentgenological changes found Invasion of adjacent bone and metastases to the lung then occurred almost simultaneously

Earlier diagnoses might have changed the prognoses especially in the last patient However the long course of the disease with few symptoms seemed to change to a malignant course rather suddenly An early diagnosis of malignancy may therefore be very difficult to evaluate even histologically

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THERMOGRAPHY IN JUVENILE RHEUMATOID ARTHRITIS

By

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Summary In the present investigation thermography turned out to be a very useful tool for examination and follow up study of child patients with rheumatoid arthritis The series comprised 46 patients with juvenile rheumatoid arthritis (JRA) and 50 non rheumatic children of the same age groups

The present investigation deals with the possibilities of thermography in examining child patients with rheumatoid arthritis Thermography findings were compared with clinical and radiological findings in the joints of 46 JRA patients In addition a thermography examination was carried out of the joints of 50 non rheumatic children who thus served as a control group

According to the examination with non rheumatic children the difference in temperature was considered pathological when it exceeded 1 0°C in symmetrical joints

Coldness of the peripheral parts of the fingers was noted more often in the thermograms of JRA patients than of the control subjects Coldness appeared to be a symptom distinctly connected with the active phase of the disease

As to the activity of the disease the thermography finding corresponded well to the clinical finding When the individual joints

were examined the thermography study was better than the clinical examination in as many as 39 % of the cases In these 39 %, distinct pathological differences in temperature were noted even before clinically manifest active joint inflammation symptoms appeared

Rheumatoid arthritis is a disease which generates excessive heat near the surface of the body and therefore thermography is a valuable method for quantitating inflammation in this disease (2 3) Results obtained from the earlier investigators (4) indicate that thermography can be a valuable tool for assessment documentation and quantification of the clinical course and response to treatment of rheumatoid arthritis *Haberman et al* (4) *have in addition revealed the important feature* that a joint may be thermographically positive before the inflammation becomes clinically manifest Thus the thermogram can also have prognostic importance

Child patients form a group of their own among the rheumatoid arthritis patients because their subjective opinion about the severity of the inflammation in the joints cannot be used in assessing the possible effect of treatment In addition there are many children with JRA who do not complain of pains in the joints though in an objective study the joints are clearly diseased (6) An insidious inflammation may be much more difficult to state with children than with adults Therefore it is understandable that thermography is received with pleasure as a new method for assessing the severity of the inflammation and for permanent registering of each situation

MATERIAL

The series consisted of 46 children with juvenile rheumatoid arthritis The diagnose *juvenile rheumatoid arthritis* (JRA) was made as proposed by Laaksonen (5)

1) polyarthritis affecting two or more joints
2) *course of a minimum of three months*
3) if only one joint is involved a synovial membrane biopsy shown changes compatible with a diagnosis of rheumatoid arthritis is desired
4) elimination of other articular or connective tissue diseases
5) onset of disease below age 15 years

These diagnostic criteria are by the way the same as proposed by Ansell Bywaters and Lawrence (1) The only patients included althou

TABLE I

Distribution of the Examined Joint Pairs

Knee	59
Ankle	50
Hand	41
Elbow	5

not complying with the criteria mentioned were those whose inflammation of the joints involved only two or three joints but had lasted at least three months

Thirty eight of the patients were girls and eight boys The objects examined were the knees ankles hands and elbows altogether 155 joint pairs were examined (Table I) Thirteen of the patients were admitted to the examination more than once The age of the patients varied from 2 to 15 years (Table II)

The control series comprised 50 children the age distribution was the same as in the investigation material A thermogram from the hands knees and ankles was taken of each child in the control group These children were admitted to the Children s Hospital of Turku University (Turku Finland) for some other than rheumatic diseases (e g epilepsy or recurrent pyelonephritis)

METHODS

An AGA THERMOVISIO Model 652 was used in the present investigation The pictures were taken of the lying patient through a silver plated mirror In order to make the examination as easy as possible the patient lay on a table in the middle of the room The examination

TABLE II

Age of the Patients Examined

Age (years)	Number
3— 7	17
8—10	10
11— 5	19

room was as free from draughts as possible (3) The pictures of the stretched ankles and knees were taken from the front the symmetrical objects being at about the same distance from the camera The pictures of the hands were taken dorsally with the hands lying side by side on the stomach on an isolating cardboard This cardboard was changed before every examination in order to avoid the harmful effect of heat from the hands A gray shade picture and an isoterm series were taken by a polaroid camera of each object Thus the warm areas and heat figures of the isoterms could be seen directly

Following previous investigations (2) the limbs were kept unclothed for a period of 10—15 minutes before the examination in a room which was maintained at a temperature of 20—22°

RESULTS

On the basis of the clinical examination 35 patients were observe to suffer from active rheumatoid arthritis and in 11 cases the diseas was in an inactive phase The disease was considered inactive in cas there were no clinically manifest active inflammation in the joints ESI was less than 15 mm/hour and CRP was negative Furthermore i could be noted that hypergammaglobulinemia in electrophoresis an elevated $alpha_2$ fraction were most often apparent in the active cases

In the control group the thermography examinations showed that temperature differences varied from 0—0 5°C in the symmetrical joints On the basis of this the findings can be considered pathological in child patients if temperature differences in the symmetrical joints are more than 1 0°C

In a normal thermogram the gray shades are as well as the isoterms among themselves rather similar (Fig 1) As to inflamed joints the thermograms are asymmetrical which is clearly indicated by the isoterms This can be seen in the thermograms of the knees in fig 2 The temperature differences between the diseased and correspondent healthy joints vary from 1 to 5°C The result was checked by a bolometer and skin thermometer in several cases (Fig 7)

In pathological cases increase of temperature most often starts from the medial side of the knee and then the inflammatory process spreads over the whole knee (Fig 3) Corresponding regular increase of temperature could not be noted in the other joints examined

TABLE III

Thermography Findings are Correlated with Radiological Findings and Clinical Activity of the Disease

	Pathological		Normal		
	Number	%	Number	%	
Active	35	100	0	0	Thermography
35 cases	24	69	11	31	Radiological finding
Inactive	4	35	7	65	Thermography
11 cases	7	65	4	35	Radiological finding

In the thermograms of the knees and ankles the borders of the warm areas often correspond to the joint surfaces This is shown by figs 4 and 5 On the basis of this it can be thought that thermography registers besides heat radiation caused by hyperemia also radiant energy caused by elevated metabolism of the synovial tissue typical of RA The curve of the local area of the joint must not be forgotten the middle is nearest the mirror and thus gives the impression of being hottest if this is interpreted as a pathological change it can cause fallacious conclusions

Haberman et al (4) observed in their investigation that adult patients with RA have often prominent venous patterns in the thermograms The corresponding phenomenon could not be noted in child patients On the contrary the increased temperature of the joint easily covered the possible normal venous pattern As few as two children with RA were observed to have figures in thermograms which could be explained as venous stripes (Fig 9 c) On the other hand attention was paid to coldness in peripheral parts of the fingers of children with rheumatoid arthritis Distinct coldness was apparent in 19 pictures of the hands 15 of these children were in the active phase of the disease In the control group coldness of fingers could be found only in five cases moreover the preliminary diagnosis of one of these was dystonia neurocirculatoria Fig 6 shows clearly cold fingers in the hands of a child with active RA

A pathological thermography finding was noted in all active rheumatoid arthritis cases (Table III) and in four cases which were



Fig 1 Normal thermogram a) and b) of hands c) of knees d) of ankles

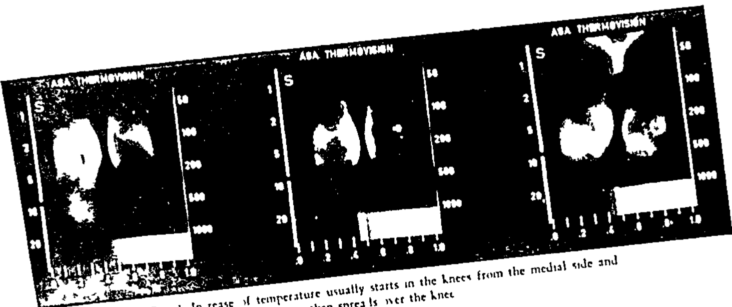


Fig 3 Increase of temperature usually starts in the knees from the medial side and then spreads over the knee

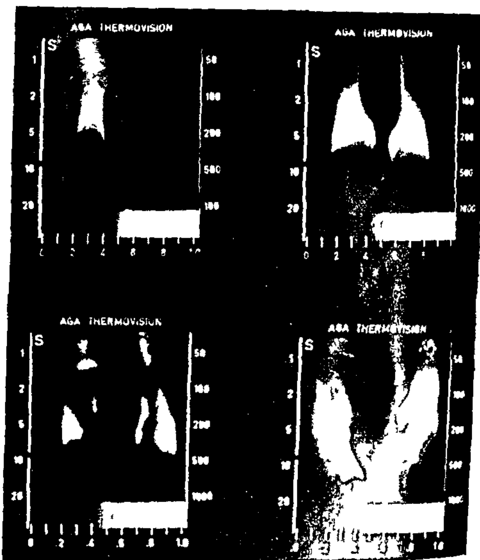


Fig 5 Pathological thermograms of ankles

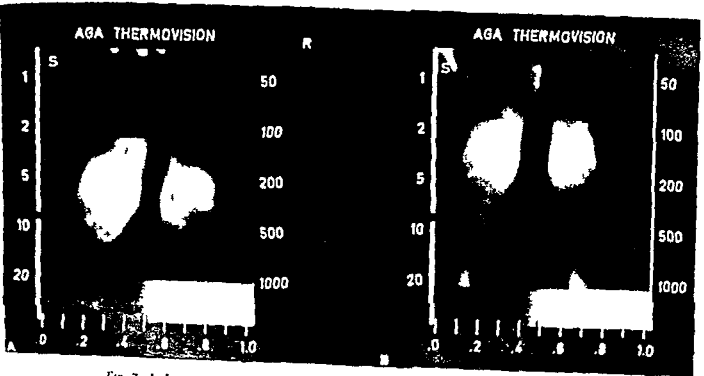


Fig 7 A four year old girl with rheumatoid arthritis Clinically *the right knee is inflamed* a) In therm graphy both knees are h t a stronger increase of temperature in the left ... Contr l examinations by therm graphy b lometry and skin thermo e same re ults b) After 1 month even the *left knee was clinic lly* ...

Fig 9 *An eleven year old boy* a)
Both wrists to the left *I—II* and to
c) Pathological thermograms in the l
the right knee) d) In the ankles and
tar

TABLE IV

Correlation of Clinical Joint Symptoms and Thermography Findings of Individual Joints

Clinical symptoms	Thermography	Number	%
+	+	25	55
—	+	18	39
+	—	3	6

clinically classified as inactive. These four patients however developed an even clinically active phase in some weeks. This explains the thermography finding and strongly emphasizes the prognostic significance of thermography (Fig. 7).

Positive radiological findings (periostitis, local growth disturbances, narrowing of joint spaces, erosions and fusions) were almost equally common both in the active and inactive phases.

In repeated thermography examinations the course of the disease and the effect of treatment could be followed. An example of this can be seen in fig. 8, which shows that strong increase of temperature has been reduced again to almost normal two weeks after an intra-articular injection of corticosteroid.

When the clinical joint symptoms were compared with regard to individual joints with thermography findings the following facts could be observed (Table IV): thermography corresponded to clinical findings in 25 cases (55 %). In 18 cases (39 %) thermography clearly gave a pathological finding before clinical joint symptoms appeared. In three cases clinical examination revealed an active finding in a more convincing way than thermography.

DISCUSSION

The present investigation shows that thermography is a valuable tool for following the course of juvenile rheumatoid arthritis. The authors made the general observation however that interpretation of the pictures is difficult and easily leads to fallacious conclusions unless the same person both takes the pictures and interprets them. Thermograms of knees can be interpreted rather easily but as to hands and feet it i

difficult to localize the hot areas solely with a picture as is shown e g by fig 9

If thermography is carried out carefully it offers excellent material to clinicians to follow objectively the course of the disease Activization of the disease can be prognosticated when the changes in the thermograms are followed

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OBSERVATIONS ON THE SYMPTOMS AND SIGNS OF EARLY RHEUMATOID ARTHRITIS IN A PROSPECTIVE STUDY

By

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Summary The authors have made a detailed study of 202 cases recognized as early RA All of them were referred to the Institute for thorough examination and were afterwards followed up for two to six years The observation of the course of the disease revealed four groups 1 Cases where development of bone erosions proved the diagnosis of RA 2 Cases in whom all previous symptoms and signs disappeared 3 Cases in whom diagnosis could not be established after follow up 4 Cases in whom other diseases were manifest The first two groups were analysed retrospectively to find the characteristic features in the early stages of RA

This study carried out since 1962 by the physicians of our Department of Rheumatology intends to present investigations on early clinical symptoms and signs in RA

The term early rheumatoid arthritis may have two meanings

1) either it means cases of short duration of the disease (e g up to one year) or
2) cases with definite clinical and radiological manifestations suggestive of RA but without advanced radiological changes particularly with no erosions of bones so characteristic of late stages of the disease Of decisive importance in the diagnosis of such cases is however a longer follow up observation confirming the progress of the disease

and particularly development of bone erosions in later periods of the disease

As most of the patients cannot give an accurate date for the onset of the disease we thought it more logical and justified to adopt the second concept of early rheumatoid arthritis

MATERIAL STUDIED

All the patients with joint pain and swelling of at least three months duration suggesting the diagnosis of RA were referred to the ward of the Institute of Rheumatology by out patient clinics for hospitalization and thorough examination of their clinical and laboratory signs

In the Institute the diagnosis was based on the criteria of A R A (3 4) recommended for epidemiological use by the Symposium organized by C I O M S in Rome in 1963 (2) At least 4 criteria were taken into consideration As an obligatory condition of the choice of cases for the group investigated the rule was accepted that no radiological changes should be present on examination of hands feet and all the joints clinically affected or there existed only doubtful radiological changes i e categories 0 and 1 according to the Atlas of Standard Radiographs of Arthritis (1)

TABLE I

Material Studied

	RA	n[illegible]n RA	D[illegible] not estab[illegible]	M[illegible] other [illegible]	[illegible]
Number of samples	[illegible]	41	1	16	[illegible]
Males females	[illegible]	[illegible]	[illegible]	9	[illegible]
Age range	17—71	15—69	16—[illegible]	15—[illegible]	15—[illegible]
Age till 0 (yrs)	9	8		1	[illegible]
1—40	40	1	10		[illegible]
41—60	55	5	5	4	[illegible]
61→	0	7	4	4	
Probable duration of the disease					
<1 yr	56	6	13	7	[illegible]
1— yrs	6	3	3	5	[illegible]
2—5 yrs	3	3			[illegible]
>5 yrs	19	9			

* see tab III

All patients belonging to the exclusions groups have been eliminated if signs of exclusions were found during the first hospitalization

In this way we obtain a group of 202 adult patients (58 men 144 women) who were followed up two to six years

During the first admission to the Department probable definite or classical RA could be recognized according to the criteria

TABLE II

No of Cases of Probable Definite and Classical RA

	At the first examination no of patients		
	seronegative	seropositive	total
Probable RA (4 crit)	31	6	37
Definite RA (5—6 crit)	77	38	115
Classical RA (7—8 crit)	1	49	50
	109	93	202

FURTHER PROCEDURE

During the observation in the hospital many diagnostic investigations were done including in addition to clinical and radiological examinations various laboratory tests which will be reported below

All patients obtained a proper treatment in the hospital usually aspirin antimalarials and gold salts were given as a basic treatment After several weeks to several months they were put under the charge of the rheumatological out patient clinics for further treatment

After the lapse of two years all patients were invited for check up under clinical conditions in order that detailed examination be conducted again and the progression of the disease determined As not all the patients reported at once the time interval of the two surveys was two to four years

If during the second hospital observation radiological examination revealed appearance of erosions of bones the patient was included in the group with recognized progression of the disease This group was called the RA group

If on the other hand the control examination disclosed a disappearance of clinical manifestations as well as of serological and radiological signs (if they were present during the first examination) the patient was

followed up further If no clinical manifestations and no progress of radiological signs were observed during 4—6 years the patient was *included in the group called non RA group*

Of course it cannot be forecast with all certainty that these patients will not experience a recurrence of articular pain and swelling in future even with development of the full classical syndrome of RA but throughout the whole period of observation they could be regarded as healthy

In this way, after several years of follow up observation we selected 4 groups of patients

124 with development of bone erosions confirming the diagnosis of erosive inflammatory polyarthritis

41 in whom all previous symptoms and signs disappeared

21 in whom a sure diagnosis could not be established after follow up

16 in whom other diseases were manifest

TABLE III

The Results of the Second Examination after 2—4 Years of Probable Definite and Classical Cases of RA

	Number of cases						
	prob	%	defin	%	class	%	total
Progress of disease (RA group)	10	27 0	67	58 3	47	91	124
Full remission (non RA group)	19	51 4	21	18 3	1	-	41
Diagnosis still not established	5	13 5	15	13 0	1	[illegible]	1
Manifest other diseases	3	8 1	12	10 4	1		16
	37	100 0	115	100 0	50	100 0	0

The results of the second examination evaluated in percentages revealed the following data

Progress of the disease in the group of probable cases was noted in 27 % in definite — in 58 3 %, in classical — in 91 %

Full remission subsisting up to 5—6 years was observed in 51 4 % of probable cases in 18 3 % of definite and only in 2 % of classical cases

In our further consideration we analysed in retrospection the symptoms and signs which were present in the early period of the disease in both RA and non RA groups and a comparison was made in order to find the characteristic features of the RA group

RESULTS

In group I with the diagnosis of RA the following features were found to be statistically significant

TABLE IV

Statistically Significant Features of RA

	RA (no. of cases) 124	non RA (no. of cases) 41	Significance of difference (P)
Subcutaneous nodules	16	0	<0.07
Muscular atrophy in hands	69	6	<0.001
Lymphadenopathy (axillar cubital)	79	19	<0.05
Waaler Rose test positive	81	2	<0.001
Punched out areas in x rays	51	2	<0.001
Anemia*	58	10	<0.01
CRP positive	98	20	<0.001

* M Hb <75 % F Hb <70 %

History significant of RA

TABLE V

History Significant of RA

	RA 124	non RA 41	
Onset insidious	65	11	P<0.01
At onset involvement of hands or/and feet only	86	11	P<0.001

On the other hand the following features were equally frequent in both groups

TABLE VI

Signs and Symptoms Equally Frequent in Both Groups

	RA 124	non RA 41
Morning stiffness	87	4
Pain in one joint	124	41
Swelling of one joint	123	41
Swelling of another joint	122	38
Symmetrical joint swelling	109	34
Mean no. of swollen or limited joints	6.6	5.6

TABLE VII

Features Equally Frequent (Not Specific) in Both Groups

	RA 1 1	n n RA 41	P
Nutritional impairment	20	6	>0 05
Weight loss	30	7	>0 05
Moist palms and feet	64	25	>0 05
Erythrosis palmaris	35	14	>0 05
Osteoporosis	49	13	>0 05

TABLE VIII

Laboratory Signs Equally Frequent in Both Groups (Not Specific)

		RA 1 1	n n RA 41
ESR elevated	20—50 mm/1 h	48	16
	above 50 mm/1 h	47	9
Leucocyte count	below 5 000/1 ml		8
	above 10 000/1 ml	[illegible]	3
Eosinophils above 5 %		23	7
Monocytes above 5 %		37	11

TABLE IX

Serum Protein Content (Not Specific Alterations Equally Frequent in Both Groups)

	RA 1 1	non RA 41
Total protein content diminished	40	19
Albumins diminished	28	3
Globulins — alpha 1 elevated	[illegible]	8
Globulins — alpha elevated	5	18
Globulins — beta elevated	10	
Globulins gamma elevated	48	11
Seromucoid content elevated	10	31

CONCLUSIONS

The observation of the course of the disease in early cases of RA treated with traditional methods revealed that

1) Pain swelling and symmetrical joint swelling can be considered

as the essential symptoms and signs suggestive of early RA although they appeared with the same frequency in both groups

2) As additional symptoms and signs characteristic of RA and suggesting a further development of the disease towards a full clinical syndrome of RA may be considered

subcutaneous nodules
muscular atrophy in hands
lymphadenopathy
sheep cell agglutinating test positive
punched-out areas in x rays
anemia
frequent appearance of CRP
at onset involvement of hands or/and feet only

3) In the early stages of the disease the activity is not as important as the above mentioned criteria

4) The existence of seven and eight criteria may be considered as an indication that further radiological and clinical changes will occur In such cases traditional methods of treatment were not sufficient to assure remission

5) Only the exact estimation of the clinical radiological and biological state of so called early cases will allow comparable results of treatment

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THE PREVALENCE OF RHEUMATOID ARTHRITIS IN OCCUPATIONAL GROUPS

By

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Summary Distributed on 66 large occupational groups the prevalence of classical, definite, probable and possible RA (according to ARA criteria) was determined in the general population A total of 39 418 persons were investigated in a sample survey of five geographical areas in Sweden — this being the total populations over the age of seven The distance between the different areas is 150—650 km Occupational groups with high prevalence of RA were Males Food and dairy workers, butchers, *fishermen* agricultural workers building *foremen* machine and engine repairers, bakers *foremen* washers and ironers *textile* workers, *industry* and factory workers. Females Cleaning workers doctors and nurses, nurse assistants, textile workers shop assistants bank post and telegraph personnel and wives

From the present series it seems as if *outdoor occupations* with relatively heavy work have the highest prevalence of RA Environmental factors apparently account for differences in prevalences of RA

The aim of this investigation was to ascertain whether or *not* any particular type of occupation may predispose *to a higher* or *lower* prevalence of rheumatoid arthritis

Detailed information on the prevalence of RA *in occupational groups* in total populations is sparse and that which is available is often not satisfactory because the occupational distribution is not correlated to the population at risk

In connection with an epidemiological study on rheumatic diseases the prevalence of RA (defined according to the ARA criteria (18 11)) *was registered in total populations in Sweden (39 418 persons in all)* The prevalence of RA in occupational groups shall here be accounted for

MATERIAL AND METHODS

Technical procedures including sampling techniques plan of collection of data a detailed investigation routine practical aids in the investigation as well as calculation methods statistically and by computer are described elsewhere (1 7) The characteristics of the population investigated including density of population in the different regions Swedish civil registration individual statistics trade within the areas and geography climate geology vegetation and water supply of the areas in which the populations live have been reported in every particular (7)

The reliability and validity of the investigation regarding diagnostic methods methods of investigation statistical methods and computer technique is discussed (7)

1 *Populations Investigated*

A *Total general populations* over the age of seven were examined as to skin and rheumatic diseases in the following five major regions in Sweden — in the counties of a) Norrbotten 8 897 persons (3 916 males and 4 981 females) b) Jamtland 3 302 persons (1 662 males and 1 640 females) c) Skaraborg 10 465 persons (5 258 males and 5 207 females) d) Kristianstad west 7 382 persons (3 613 males and 3 769 females) e) Kristianstad east 5 287 persons (2 571 males and 2 716 females)

B *Special populations* were also included in the study

a) Steel workers in a steel works in Norrbotten 1 298 males
b) Prisoners in the Open Borstal Institution of Ulriksfors Jamtland 99 males
c) Males undergoing medical examination on enlistment in the enrolment area of IO 3 (parts of the counties of Örebro and Vastmanland) 825 males
d) Males in the defence forces refresher training course (Revinge and Hassleholm Kristianstad west) 721 males

e) Re investigations in Norrbotten and Kristianstad 1 142 persons (48? males and 660 females)

The distance between the northernmost and southernmost area is about 1 500 kilometer The collected material is considered to be good national geographical sample

2 *Diagnostic Criteria*

Active rheumatoid arthritis All persons were investigated (naked or seminaked) by the same doctor Aids used in diagnosis were hospital records x ray as well as out patients records Useful symptoms were 1/ morning stiffness 2/ pain on movement or tenderness in at least one joint 3/ swelling of soft tissue or fluid in at least one joint 4/ swelling of at least one other joint 5/ symmetrical joint swelling with simultaneous involvement of the same joint on both sides of the body (NB terminal phalangeal joint involvement does not satisfy this criterion) 6/ subcutaneous nodules 7/ x ray changes typical of RA e g decalcification degenerative changes are not acceptable 8/ positive agglutination test If three or four criteria were fulfilled this was defined as probable arthritis five or six definite more than six classical possible arthritis if two criteria in (18) were fulfilled and joint symptoms during at least three weeks Primarily in the investigations the first six criteria in (11) and (18) were used When hospital records x ray data etc were available criteria 7 and 8 in (11) were used

Inactive rheumatoid arthritis Cripples with inactive arthritis may fail to fulfil the above mentioned criteria and others had therefore to be used viz 1/ a past history of polyarthritis 2/ symmetrical deformity in peripheral joints consisting of *ankylosis irreducible subluxation some involvement of one hand or foot* 3 x ray changes of RA 4/ positive serological tests for rheumatoid factor Definite meant that three or four criteria had been fulfilled probable two criteria fulfilled (11)

Exclusions according to the ARA criteria (11)

Criteria used for ankylosing spondylitis and arthritis psoriatica as well as codes for these diseases and RA are in detail accounted for in (7)

The diagnosis of RA was based on macro-morphological objective findings from joints muscles and tendons often completed with data from hospital records and x ray documents A history of rheumatic diseases including family history past history mode of onset course of the disease previous hospital care x ray results was taken Physical findings were drawn on outlines of bodyform using the Jansen s system (8) Most patients with RA were re examined and the history completed after the end of the routine investigations

3 *Occupational Classification*

The principles for grouping of occupations the computer ... and occupational groups, are in detail accounted for in ( )

Definition of occupation Individual occupation is the work done by an individual worker and only exceptionally does it contain information on how people work the methods used and never data on education and other qualifications

Occupational family is the grouping together of individual occupations with similar types of work (sales representatives)

Occupational groups is the grouping of similar individual occupations and occupational families involving special type of work, i e technical work, road work, wood work, etc

The general principles of classification of occupations was to bring together those individual occupations which could be grouped together as according to the work performed without consideration of education employment status rank or competence of the individual In the main therefore no differentiation was made between workers with simple duties and those with skilled work of a similar kind and no simple principle for judging any similarity between occupations could be followed.

All persons investigated were asked for their occupation Occupations were also controlled at the place of working at the time of examination

4 *Statistical Techniques*

Sampling methods Total sampling technique was chosen for the primary investigation of the populations over the age of seven All people within a geographical area chosen for the survey had to be investigated They passed seminaked through an examination room Systematic random sampling was used for the groups (10—70 %) not responding to the call for the primary investigation Be and for the groups defined in Bc above

Calculations When comparing the prevalence of RA between different occupations the disturbing influence of irrelevant background factors such as age must be eliminated The standard population method (10) was used to calculate standardized index numbers which constitute directly comparable units The standard population was in this case the total number of persons investigated (39 418) Apart from these index numbers the computer programmes have produced lists of the percentage distribution of RA in different occupational groups

A detailed account for the mathematical aspects of the study has been given elsewhere (7)

RESULTS

Tables I—IV show the prevalences of classical definite probable and possible RA in occupational groups for males females and both sexes together The total numbers of patients with RA are given in the tables together with percentage and standardized indices The reliability of the indices is high as the subgroups are rather big (range generally 100—6 000 persons in each occupational group)

The ranking order of the prevalences of RA in occupational groups according to the indices differs in some respects from the percentage

figures Thus differences in the age distribution in different occupational groups may lead to false ranking order of RA frequency if the standard indices are not used Ranking of some prevalences of classical definite probable and possible RA according to the indices from the highest to the lowest prevalence in the occupational groups is shown in tables V–VIII

In the structural correction calculations three age groups were used (0—29 years 30—49 years 50—99 years) (1)

It seems as if the prevalences of RA (classical definite probable possible) in male occupational groups such as food and dairy workers, butchers fishermen agricultural workers building foremen machine and engine repairers bakers foremen washers and ironers, textile workers industry and factory workers (table IX) are comparatively high For females Cleaning workers doctors and nurses nurse assistants textile workers shop assistants bakers bank post telegraph personnel and wives with domestic work have comparatively high prevalences of RA

There is at the moment no explanation for the fact that RA is specially common in certain occupational groups

DISCUSSION

Literature In one series patients with RA were correlated to the occupations of the population at risk No significant differences between different occupations regarding the prevalences of RA could be demonstrated (22)

A study of the results in the light of the statistics of the Central Office of Statistics of Finland derived from the 1950 decade reveals no significant differences between the different occupational groups regarding the prevalence of RA (21)

In a study of 102 patients it was concluded that specific occupation did not play much role in the etiology of the disease (23) and in a series of 573 patients it was similarly concluded that RA attacked all social classes and that relatively light indoor work almost appeared more predisposing than heavy outdoor work (25)

In a study of 1 000 arthritic patients in England relatively more RA than other forms of rheumatism was found among indoor workers with the possibility remaining that the arthritis had altered the patients occupations in some instances (13) In two other series (1 9) a hi

proportion of patients with RA was found among males with indoor rather than outdoor occupations while the reverse finding was obtained in a third (23 24)

In one series 41 % were obliged to change their employment as a direct cause of the disease in all cases seeking either lighter work or work with more suitable hours or working conditions (17) which possibly could explain the high prevalences among indoor occupations Most rheumatoid arthritics however can do regular work (5) A greater proportion of persons working under stress had RA in one series (4)

A relatively low frequency of RA has been reported among miners (3 9) In a recent epidemiologic survey (16) the frequency of RA in miners approximated that in males engaged in other occupations Men engaged in mining had a high incidence rate of arthritis (11 3/1 000) and this rate was significantly higher than for men engaged in the professional clerical manufacturing and transport and public service groups but not significantly greater than for those employed in building and road making or distributive trades (2)

In a series of 388 cases of RA in England the disease was shown to be common among domestic duties casual labouring work retail trade ploughmen miners (20)

It has been claimed that there is a causal connection between trauma and RA and that the disease is especially common among manual workers stone breakers mechanics wood cutters farm labourers because the risk of trauma is greater in these occupations than in many others (19) In one series it was found that building workers and stone workers were 30 % of all male occupations with RA (15)

In a study of invalids in Sweden 1933 it could be demonstrated that from the total numbers of farm workers who were invalids 15 6 % of the males and 14 8 % of the females were rheumatoid arthritics Proportional figures for other occupations were Farmers males 13 7 % females 18 8 % forest workers males 13 7 % fishermen males 11 9% traffic workers males 10 4 % unskilled workers males 11 8 % females 17 8 % industry and factory workers males 9 2 % females 8 9 % domestic workers housewives females 12 3 % (6)

In every occupational group in which both men and women are represented in substantial numbers the age adjusted prevalence rate for females is higher than that for males (26)

In most studies in the literature no associations between the prevalence of RA with the occupations have been noted (12)

The results of the present series show that the prevalences in different occupational groups in total populations differ considerably In most of the series from the literature it is not possible to compare the occupational distribution of patients with RA with that of the population from which they were drawn This might explain that no differences between occupational groups can be proved The results presented here show in some respects similarities with some of the series in literatur (6 15 19 20) in which comparatively high prevalences of RA were found among male workers in agriculture fishing and building industry and females in domestic work and housewives In the present series the prevalence of RA was in the average higher among outdoor workers than among indoor workers in accordance with (23) and (24) It seems as if male workers outdoors with rather heavy work have the highes prevalences of RA Bakers fishing industry workers food and dairy workers butchers textile workers show high prevalences of rheumatoid arthritis among both males and females From these data it seems probable that there are one or more factors of environmental kind (i e infections) taking part in the pathogenesis of RA

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Prevalence of Classical Rheumatoid Arthritis (RA) in 66 Occupational Groups (Males Females Both Sexes) Total Populations of 39 418 Persons Investigated

Occupation	Total (Males + Females) Index	Per cent	No RA	Males Index	Per cent	No RA	Females Index	Per cent	No RA
Nurse assistant	43 15	0 [illegible]	2	0 00	0 00	0	575 09	[illegible]	2
Office worker	177 63	0 47	6	187 39	0 44	2	176 33	0 49	4
Office worker clerk	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Painter	114 86	0 36	1	155 06	0 38	1	0 00	0 00	0
Pensioner	156 20	1 31	5	170 92	1 12	2	146 98	1 48	3
Physiotherapist	0 00	0 07	0	0 00	0 00	0	0 00	0 00	0
Plate worker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Plumber	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Precision tool maker	[illegible]00	0 00	0	0 00	0 00	0	0 00	0 00	0
Printing worker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Prisoner	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Representative agent	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Road worker chauffeur	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Shoe and leather worker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Shop assistant	1 4 16	0 35	4	106 06	0 33	1	95 07	0 36	3
Steelworker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Storeman and stock room worker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Student	1 85	0 01	1	5 13	0 03	1	0 00	0 00	0
Teacher	342 86	0 93	5	185 69	0 44	1	336 14	1 10	4
Technician	53 8	0 29	1	79 87	0 30	1	0 00	0 00	0
Textile worker	65 09	0 21	1	0 00	0 00	0	75 00	0 30	1
Traffic worker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Various writing work	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Washer and ironer	0 00	0 00	[illegible]	0 00	0 00	0	0 00	0 00	0
Watchman	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Wife	99 49	0 33	37	0 00	0 00	0	80 78	0 39	37
Woodworker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0

TABLE II

Prevalence of Definite Rheumatoid Arthritis (RB) in 66 Occupational Groups (Males Females Both Sexes) Total Populations of 39 418 Persons Investigated

Occupation	Total (Males + Females)			Males			Females		
	Index	Per cent	No RB	Index	Per cent	No RB	Index	Per cent	No RB
Nurse assistant	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Office worker	176.17	0.71	9	179.10	0.66	3	86.48	0.73	6
Office worker clerk	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Painter	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Pensioner	123.78	2.62	10	79.36	1.12	2	142.92	3.94	8
Physiotherapist	226.43	2.15	2	0.00	0.00	0	187.12	2.70	2
Plate worker	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Plumber	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Precision tool maker	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Printing worker	107.56	0.91	1	185.40	0.98	1	0.00	0.00	0
Prisoner	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Representative agent	90.15	1.04	2	16[illegible].23	1.13	2	0.00	0.00	0
Road worker chauffeur	31.34	0.31	2	51.57	0.32	2	0.00	0.00	0
Shoe and leather worker	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Shop assistant	109.66	0.52	6	148.58	0.33	1	84.04	0.59	5
Steelworker	80.63	0.47	6	125.23	0.47	6	0.00	0.00	0
Storeman and stock room worker	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Student	0.73	0.01	1	0.00	0.00	0	1.01	0.03	1
Teacher	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Technician	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Textile worker	183.96	1.[illegible]9	6	464.12	2.34	3	97.15	0.89	3
Traffic worker	61.47	0.73	1	105.73	0.76	1	0.00	0.00	0
Various writing work	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Washer and ironer	473.28	4.55	1	865.21	5.56	1	0.00	0.00	0
Watchman	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0
Wife	154.59	1.36	129	0.00	0.00	0	113.37	1.36	129
Wood worker	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0

O cup tion	Tot l (Males + Females)			Males			Femal s		
	I dex	Per cent	No RI	I x	Per nt	No RI	Index	Per ce t	N RI
Nurse assistant	282 [illegible]	[illegible]	[illegible]	0 00	0 00	0	[illegible]	[illegible]	4
Off e work r	97 10	0 86	11	31 50	0 22	1	93 94	1 22	10
Off ce w rker clerk	257 71	1 05	1	527 23	1 19	1	0 00	0 00	0
Pa nter	31 85	0 3(	1	54 75	0 38	1	0 00	0 00	0
Pens oner	9 1(	0 26	1	0 00	0 00	0	13 36	0 49	1
Physiotherapist	83 81	1 08	1	0 00	0 00	0	(9 98	1 35	1
Plate orker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Plumber	119 48	0 98	1	180 60	1 00	1	0 00	0 00	0
P ecisi n tool maker	2(9 78	2 90	2	208 84	1 67	1	1329 55	11 11	1
Printing worker	79 62	0 91	1	134 26	0 98	1	0 00	0 00	0
Prisoner	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Representative agent	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Road worker chauffeur	75 71	0 77	5	117 31	0 81	5	0 00	0 00	0
Sh e and leather worker	52 29	0 55	1	0 00	0 00	0	137 7(	2 01	1
Shop assistant	1?4 89	0 87	10	41 62	0 33	1	119 79	1 07	9
Steelworker	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
St reman and stock room worker	74 0?	0 (8	2	127 97	0 73	2	0 00	0 00	0
Student	1 09	0 03	2	0 00	0 00	0	1 56	0 06	2
Teacher	130 05	0 85	5	(2 43	0 41	1	114 83	1 10	4
Technician	32 74	0 58	2	53 70	0 61	2	0 00	0 00	0
Textile worker	119 62	1 07	5	0 00	0 00	0	122 53	1 49	5
Traffic worker	91 00	1 46	2	152 40	1 52	2	0 00	0 00	0
Var ous writing work	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Washer and ironer	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Watchman	0 00	0 00	0	0 00	0 00	0	0 00	0 00	0
Wife	156 89	1 76	167	0 00	0 00	0	115 42	1 76	1(7
Woodworker	258 97	2 47	2	408 36	2 50	2	0 00	0 00	0

... from Highest to Lowest Pre...
... Definite (RD) Probable (RI) and Possible (RT) Ra...
*Arthritis in 66 Occupational Groups A Low Number or Sum Means Hig...
lence of Rheumatoid Arthritis and Vice Versa*

Rank	Type of Rheumatoid Arthritis				Occupation
	RT	RI	RB	RA	
sums 15	41	30	30	24	[illegible]
76	24	35	9	8	Office worker
98	41	3	30	24	Office worker clerk
106	30	30	30	16	Painter
110	39	36	24	11	Pensioner
131	41	36	30	24	Physiotherapist
94	4	36	30	24	Plate worker
108	41	13	30	24	Plumber
78	12	17	30	24	Precision tool maker
91	41	18	8	24	Printing worker
121	31	36	30	24	Prisoner
75	3	36	12	24	Representative agent
98	25	21	28	24	Road worker chauffeur
131	41	36	30	24	Shoe and leather worker
95	33	33	16	13	Shop assistant
98	20	36	18	24	Steelworker
87	9	19	30	24	Storeman and stock room worker
179	40	36	30	23	Student
109	41	28	30	9	Teacher
95	13	37	30	20	Technician
69	6	36	3	24	Textile worker
99	41	14	19	24	Traffic worker
131	41	36	30	24	Various writing work
63	2	36	1	24	Washer and ironer
131	41	36	30	24	Watchman
131	41	36	30	24	Work in household
101	41	6	30	24	Woodworker



Fig. 2. Photomicrograph showing irregular endothelial tissue, exudate and black reduced osmium in the fat tissue one hour after the injection. 50 x

was observed underneath the endothelium inside the synovial membrane. One hour after the injection exudate stained like fibrine was visible under the endothelium (Fig. 2). The endothelium was irregularly arranged bordering incoherently on its basement. Osmium was



Fig. 3. Twenty-four hours after the injection the [illegible] polymorphonuclear leukocytes [illegible] immigrated endothelium and black osmium [illegible] the fat tissue. 100 x



Fig 4 Photomicrograph taken five weeks after the injection shows a layer of fibroblasts on the surface of the synovial membrane and inflammation cells deeper in the synovial tissue 750 x

detectable as black aggregates and veil like hues of the surface of the fat cells

One hour after the drug injection the synovial lining cells contained poorly defined often broken organelles which presumably reflected disintegration of the chemically injured cells There were no osmium

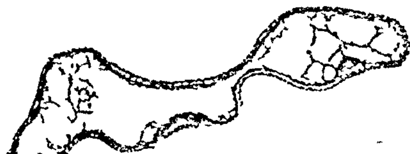


Fig 5 An electron micrograph of a fat vacuole in the deeper parts of the synovial tissue *one hour after* OsO_4 *injection Reduced* smium s seen deposited as a thin layer n the fat vacuole surface Fixed in glutaraldehyde N staining 9800 x

DISCUSSION

The results show that osmium tetroxide has a rapid possibly instant effect on the endothelial surface of the synovial membrane As early as one minute after the injection black reduced osmium appeared under the synovial endothelium The observation also supports the previously suggested view of great permeability of the synovial membrane (15) Apparently osmium tetroxide which is used as a tissue fixative in electron microscopy coagulates the synovial endothelium when in contact and consequently the tissue becomes necrotic and disappears in a few days Corresponding observations were made by von Reis and Swensson (14) Deeper in the synovial membrane the reaction is secondary A foreign body granuloma caused by osmium particles is produced and later a connective tissue scar develops The scar is observable five weeks after the injection but might develop much earlier Increase of the collagen connective tissue caused strong thickening and hardening of the synovial membrane It is possible that the articular secretion declines or ceases during this process and begins anew when synovial endothelium regenerates There was no observable regeneration during five weeks

In the present study the fine structure of the rabbit synovia in the control knees was similar to that described in the literature (1 2) Osmium tetroxide is known as an excellent fixative and it reacts with lipids and related compounds (17) and with amino acids peptides and proteins (7) The osmium deposits were encountered as bizarre shaped sponge like structures in fat vacuoles After five weeks similar deposits were surrounded by giant cells This finding supports the view that osmium tetroxide reacts predominantly with tissue lipids (15) The osmium deposits consist of stable compounds and they remain in the tissue for a long time

An intra articular osmium tetroxide injection induces giant cell formation in the synovia as does rheumatoid arthritis (6) The osmium tetroxide induced giant cells however can be differentiated easily from those found in rheumatoid arthritis with their typical electron opaque deposits

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PSEUDOXANTHOMA ELASTICUM (PXE) AND JOINT MANIFESTATIONS

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Summary A case of PXE is reported which, besides cardiac and gastrointestinal symptoms also shows recurrent joint manifestations possibly due to the basic disease Histologically the joint lesions resembled subacute synovitis to some extent

According to a series (4) based on published reports and consisting of 200 PXE cases this syndrome was most frequently associated with the following changes besides skin lesions eye symptoms calcification of peripheral arteries signs of weakened pulsation hypertension gastrointestinal hemorrhages and severe psychic disturbances

In the case to be presented below gastrointestinal hemorrhages were most prominent but there were also distinct articular manifestations

A CASE REPORT

The patient a man aged 70 and previously healthy was admitted to hospital for symptoms of cardiac insufficiency in *1961* *There was nothing* relevant in the family history About one year later he was found to have iron deficiency anemia When admitted in 1964 he had melaena and anemia Roentgen examination of the esophagus stomach and large intestine as well as sigmoidoscopy were carried out all with negative result The patient entered hospital several times during the next years



Fig 1 The PXE lesion in the skin from the neck In the middle and lower third of the dermis the elastic fibres are increased and stain intensely black. The fibres are fragmented and often swollen, occurring in irregular bundles (v Gieson and Verhoeff s orcein stain)

because of hemorrhagic anemia the heart symptoms however were relatively well compensated In spite of repeated examinations the cause of the obvious gastrointestinal bleeding remained undetermined

Joint symptoms first appeared in 1965 the proximal interphalangeal joints of the fingers being affected with swelling and tenderness on motion In 1967 the left elbow joint became extremely tender and heavily swollen with limitation of movement The tenderness and swelling of the elbow joint disappeared after about three months In the winter of 1968 there were again symptoms in the finger joints and the left knee but they disappeared after three weeks In August of the same year tenderness on motion and swelling suddenly appeared in the right wrist these changes however lasted only a few weeks In addition the patient had occasional back pain

In December 1967 a skin specimen was removed from the neck for histological study The pathologist s diagnosis was Pseudoxanthoma elasticum

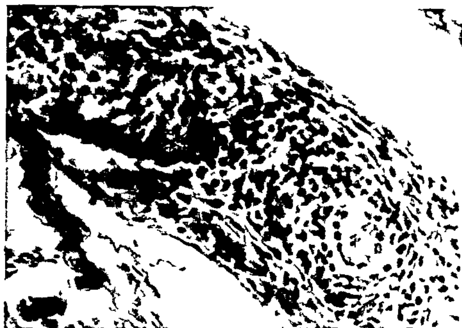


Fig. 4 Biopsy specimen from the wrist of a PXE case The synovial membrane was thickened and slightly villous There is a rich vascular pattern everywhere and the blood vessels are distended The vessel walls show mild degenerative changes and considerable cell proliferation in endothelium and perivascular tissue Inflammatory cells are few in number most cells have the appearance of lymphocytes Collagenic fibrils are swollen in part the tissue of some villi is degenerated and afibrillar The synovial membrane shows areas that have undergone hyaline fibrinoid degeneration but there is no edema worth mentioning Small areas of fibrinoid necrosis occur at the margins of the synovial membrane These are adjoined by fibrin like substance interspersed with inflammatory cell rests The tissue also contains normal and degenerated elastic fibres (v Gieson stain) No crystal forms appeared on polarization microscopic examination of a formalin fixed specimen

Skin Lesion The patient s neck and axillae showed pale yellow areas 1—2 mm in diameter resembling the skin of a hen The skin of the extremities was partly atrophic and bled easily

Eyes On repeated ophthalmological examination the eyegrounds failed to show angioid streaks whereas atrophy of the choroid was present

Heart On examination in 1961 the pulse was regular but there was occasional bigeminy There was a Grade III apical systolic murmur Blood pressure was 130/100 Hepatic and pulmonary stasis occurred Roentgenologically the heart was greatly enlarged in all directions

Pleural effusion occurred in the right lung showing in addition central stasis Peripheral pulsations were palpable

Auricular fibrillation appeared in 1966 and in addition multifocal extra ystoles In roentgenograms the heart was slightly diminished in size and changes due to stasis were nearly absent There was roentgen evidence of calcification in the arteries of the wrist but not elsewhere in the extremities

Joint Lesions The joint changes usually set in suddenly and sometimes disappeared in a few weeks There was no morning stiffness In 1968 during a hospital stay the right wrist was affected with periarticular swelling pain and tenderness on movement A specimen of synovia was taken from the wrist

The changes thus resembled to some extent the picture of subacute synovitis though the pathologist considered the finding peculiar Staining by the method of Verhoeff disclosed no appreciable changes in the elastic fibres

Roentgenograms of the hands and feet showed nothing of special interest apart from the soft tissue swelling in the right wrist Arthrotic changes occurred in the knee and elbow joints Spinal roentgenograms failed to reveal any significant findings

Certain Laboratory Studies The sedimentation rate was constantly increased varying from 30 to 50 There was also occasional leucopenia determination of leucocyte antibodies was carried out with negative result Bleeding and clotting times were within normal limits The Latex and Waaler Rose tests were repeatedly negative The same applies to the nucleus antibody test and I E cell test Immunoelectrophoresis was normal as was also urine hydroxyproline value

DISCUSSION

As recently as 1960 (8) PXE was linked with abiotrophy of the collagen tissue Indeed the skin changes affect mainly the sites most exposed to wear and tear Histochemical and electron microscopic studies carried out in the last few years seem to indicate however that in this disease it is primarily a question of injury to the elastic fibres (1 6) In the case of collagen damage the collagen matrix of bone would be expected to show changes as well Bone changes however have only

been found in cases of PXE associated with Paget s disease (10 17) Joint symptoms have been described in a case of PXE in association with Still s disease (5) and in a patient who had intermittent swellings and pain in the middle phalanges of the fingers (8) But in this last case the joints had beeen examined neither histologically nor roentgenologically Stankler (11) reported on a PXE case with swellings of the finger joints and so called knuckle pad changes

The question arises whether in the present case the joint symptoms should be kept apart from the basic disease Yet no other possible etiological factor was disclosed by the history or by the results of examination A fact arguing against hemarthrotic changes is that the synovial samples from the affected wrist showed no signs at all of previous hemorrhage Whether injury of the elastic fibres plays a part in the joint changes is a matter which remains undetermined Urine hydroxyproline value was normal suggesting no clear disturbances in collagen metabolism

The cardiac changes occurring in PXE are not specific and many investigators therefore think they can only be verified on autopsy (7) PXE is usually associated with enlargement of the heart murmurs arrhythmias unspecific electrocardiographic changes and hypertension — features which also fit this case It is interesting to note that in autopsy cases there have been changes in the endocardium similar to those occurring in endocardial fibroelastosis (7)

The disease concerned seems to be rare its incidence ratio being 1 160 000 (2 9) Reports on gastrointestinal hemorrhage in connection with PXE have been fairly few about 30 cases (3) Some of these cases probably elude diagnosis

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ON THE ETIOLOGY OF GROWTH DISTURBANCE OF THE MANDIBLE IN JUVENILE RHEUMATOID ARTHRITIS

By

EERO SAIRANEN

Summary One case of congenital micrognathia unaccompanied by any other congenital malformations was discovered among 33 000 school children surveyed This case showed roentgenological evidence of condylar aplasia The same change had been noted earlier in some cases of juvenile rheumatoid arthritis associated with micrognathia. The conclusion is drawn that in some of the children with RA associated with micrognathia this latter has a congenital basis whereas in other cases it is due to affection of the temporomandibular joint

As is well known the disturbance in mandibular growth occurring in juvenile rheumatoid arthritis (brachygnathia micrognathia, birdlike face Vogelgesicht) has been generally attributed to affection of the temporomandibular joint and the consequent effect on the condylar growth center which has been considered the most important growth center (2 4 8) *Opinions have also been advanced* according to which this growth disturbance might have another basis (3 6 22 23 24 25) Clinically a temporomandibular joint affection has not nearly always been established in cases of *micrognathia While it is* evident on the one hand that in children a joint inflammation can develop almost without being noticed (11) there are often difficulties in discovering roentgenological changes in the temporomandibular joint

MATERAL AND METHODS

A total of 33 000 school children varying in age from 6 to 15 years ere studied in an attempt to determine the frequency of micrognathia ı normal material The school nurses were at first given information n micrognathia by means of a report and a picture All doubtful cases ,ere sent by the school nurses to be personally examined by the author ı addition to clinical examination laboratory tests and roentgen studies ,ere carried out

Two children with rheumatoid arthritis who had been treated at the heumatism Foundation Hospital (Heinola) the diagnosis being onfirmed according to the commonly accepted criteria were examined y the correponding methods It may be added that chromosome analyses vere made from the peripheral blood The evaluation of the stage of he disease was made according to Steinbrocker Traeger and Batterman 1949)

RESULTS

Of 33 000 school children seven were sent for closer examination In only one of these (Case 1) was micrognathia found however

Case Report (Case 1)

The patient was a normally developed girl of 13 years who had a receding chin and malocclusion Mouth open the distance between the dental arches was 5 cm the teeth showed nothing of special interest. The temporomandibular joints were not tender The peripheral joints revealed no abnormal findings

Delivery had been normal birth weight 3 300 g length 51 cm Development in childhood as recorded by the school nurse school physician and school dentist had been ordinary At 11 years of age the girl was 146 cm long and weighed 36 kg At 8 years she had measles and at 11 years chickenpox She had been treated by a dermatologist for infantile eczema Slight hyperopia had been diagnosed and she now wears glasses

On the paternal side no diseases of special interest are known to have occurred in the family but the mother has a family history of diabetes and tuberculosis

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Fig 3 Side view (Case 1)

The father had been well if the common children s diseases are excepted The mother had had measles rubella and parotitis in childhood Because of recurrent tonsillitis a tonsillectomy had been performed in 1952 She had also been under hospital care for nervous disturbances She had had four normal deliveries All children had been healthy and well developed It should be noted that she had been in good health during her pregnancies and received neither roentgen treatment nor any medication

Orthopantomography of the girl s mandible showed condylar aplasia the condyles were as it were sawn off the temporal fossa was flat and the mandibular angle showed fairly marked notching Tomography of the temporomandibular joints revealed a corresponding condylar aplasia

Roentgen films of the cervical spine hands and feet showed nothing of special importance The Marfan index was 8 0

Blood sedimentation rate and Hbg were normal Latex and Waaler Rose tests were negative The immunoelectrophoretic pattern was normal Chromosome analysis karyotype 46 XX (normal finding)

Case Report (Case 2)

This case is described here for comparison a 13 year old boy who had had juvenile rheumatoid arthritis for three years There were no symptoms from the temporomandibular joints and no mandibular changes The stage of the disease was II

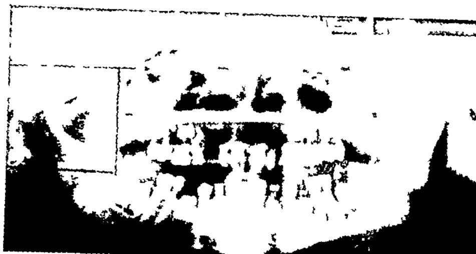


Fig 4 Orthopantomogram of the boy with RA Mandibula normal (Case ?)

Case Report (Case 3)

A 10 year old school girl is presented in whom rheumatoid arthritis developed at the age of two years There have been no temporomandibular joint symptoms Incipient micrognathia was diagnosed at three years of age The growth disturbance of the mandible later developed into definite micrognathia Serological tests for rheumatoid arthritis were negative



Fig 5 Orthopantomogram of the girl who had RA micrognathia hypoplasia of the condyle and angular notching (Case 3)

Roentgenograms of the wrists had shown erosions Chromosome analysis normal finding The stage of the disease was III

DISCUSSION

The roentgenological changes in the temporomandibular joint caused by RA seem to be most readily detected by tomography (11) or orthoradial pantomography (26) Mere flattening of the capitulum occasionally regarded as a sign of RA is not significant in this respect (12) The shape of the condyle may vary within normal limits also (17)

Aplasia or hypoplasia of the condyle and micrognathia have been found in a number of children with RA (2 16 17 25) The case here presented (Case 1) is otherwise similar but differs in that there was no RA The question arises whether such a condylar change in juvenile RA should be regarded as primary or secondary in other words as due to RA or not Martel considered this change secondary in a patient with RA since the fossa temporalis was normally developed In the cases here reported (1 and 3) the temporal fossa appeared fairly flat though evaluation presented difficulties owing to the roentgenographic technique used Indeed the development of the temporal fossa seems to depend upon a variety of factors (19)

The condylar region being a growth center which affects the height of the mandibular ramus in particular (8 27) it is probable that condylar aplasia or hypoplasia are of importance in the development of micrognathia This is also suggested by the present case (Case 1) Micrognathia may be due to various causes (28) Particular attention has been called to the part played by the masseter and the pterygoideus in mandibular modelling (5) There may be micrognathia in association with several congenital malformations but no data are available on condylar changes in these cases (7) In gargoylism on the other hand condylar changes as flattening has been noted and this has been thought to be a specific finding (10) While micrognathia is not known to occur in gargoylism it has been demonstrated in osteogenesis imperfecta The two diseases mentioned are heritable disorders of connective tissue (18) Isolated cases of congenital micrognathia unaccompanied with other changes have also been described (11) The present case most probably belongs to that group

It may be asked whether this condylar aplasia or hypoplasia when

associated with micrognathia is a sign of some genetic change related to JRA Apparently the shape of the maxilla is considerably affected by genetic factors Mandibular development and modelling however is largely dependent upon external factors (9) Genetic factors have not been definitely shown to play a part in the origin of RA (15 20) The present study revealed no data in favour of genetic factors and further studies seem desirable

Congenital micrognathia does not generally become manifest until the age of 3—6 years Some series of JRA including a relatively great number of cases with micrognathia (6 15 21 22) have shown that the disease in most of these cases had started before the age of six years It is possible then that the temporomandibular joint changes ascribed to RA included cases that were in fact congenital

In Sweden the incidence of JRA has been stated to be about 8 per 100 000 children under 15 years (13) The frequency of micrognathia varies in different series from 4 % to 50 % (23) Assuming this frequency to average 20 % a total of 100 000 children would include one to two micrognathia cases with RA On the basis of the present study there are about three children with micrognathia among a total of 100 000 The incidence is thus slightly higher than for children with RA

Obviously the micrognathia occurring in JRA is due to affection of the temporomandibular joint In some cases however the micrognathia should be considered congenital The question of how great a proportion of all cases of micrognathia have a congenital basis remains undetermined for the present

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STUDIES ON THE POSSIBLE PRESENCE OF THE URATE BINDING ALPHA 1 ALPHA 2 GLOBULIN IN HUMAN ERYTHROCYTES

By

J O ALVSAKER

Summary Recent investigations indicate that urate is partly transported through the erythrocyte membrane by a process which might involve a protein carrier system In the present work erythrocyte proteins have therefore been studied with special emphasis on the possible presence of the urate binding alpha 1 — alpha 2 globulin The erythrocytes were destroyed by freezing and thawing and the ghosts subsequently treated with desoxycholate or ultrasound The three different protein fractions obtained were subjected to anion exchange chromatography on DEAE Sephadex columns according to the procedure employed for isolating the urate binding alpha 1 — alpha 2 globulin In this way 10—14 different non hemoglobin protein zones could be distinguished by vertical starch gel electrophoresis None of these proteins however seemed to be identical to the urate binding alpha 1 — alpha 2 globulin

Evidence suggests that urate is partly transported through the human erythrocyte membrane by a process different from diffusion (6) The exact nature of this process is still obscure A carrier transport system common to several purines has been proposed (5)

Based on reports of a decreased secretion of urate into urine and saliva in gouty as compared to non gouty individuals Wyngaarden (14) mentions the possibility of a more widespread defect of urate transport

in this disorder Recently the presence in human plasma of a urate binding alpha 1 — alpha 2 globulin defective in some patients with primary gout was reported by the author (1) The possibility therefore existed that this protein besides serving as a transport protein for urate in human plasma also might be involved in the transport of urate across cell membranes Consequently investigations on the possible presence of the urate binding alpha 1 — alpha 2 globulin in human erythrocytes have been carried out

MATERIALS AND METHODS

Human Erythrocytes Erythrocytes from 500 ml blood (one healthy blood donor) were washed three times with 0 11 M sodium phosphate buffer pH 7 containing 0 1 per cent (w/v) of glucose (total volume 5 l) The washed erythrocytes were resuspended in 240 ml of physiological (0 9 per cent w/v) NaCl solution and stored at —20° C

Column Materials Sephadex G 50 coarse grade and DEAE Sephadex A 50 medium grade were purchased from AB Pharmacia Sweden

Supporting Media for Electrophoresis For immunoelectrophoresis Special Agar Noble purchased from Difco Laboratories USA was employed whereas for vertical starch gel electrophoresis Starch Hydrolysed from Connaught Medical Research Laboratories Canada was used

Anti sera Horse immune serum against human serum (Centraal Laboratorium Van De Bloedtransfusiedienst Van Het Nederlandsche Rode Kruis), anti prealbumin serum anti alpha 1 lipoprotein serum anti alpha 2 lipoprotein serum anti beta lipoprotein serum and anti alpha 2 macroglobulin serum purchased from Behringswerke AG Germany and rabbit immune serum against the urate bindning alpha 1 — alpha 2 globulin prepared as previously described (1) were used

Preparation of Erythrocyte Protein Fractions

A The erythrocyte suspension was thawed at room temperature centrifuged at 1 000 × g for 15 minutes and the supernatant was sucked off

B *To part of the residue containing the ghosts (80 ml)* was added 0 01 M sodium phosphate buffer pH 7 35 (80 ml) containing sodiumdesoxycholate (Mann Research Laboratories) to a concentration of 1 per cent (w/v) (4) After 30 minutes (at +4° C) the solution was centrifuged at 1 000 × g for 15 minutes (at 0 C)

C Part of the residue containing the ghosts (33 ml) was exposed to ultrasound for 30 minutes with ice cooling (MSE Mullard Ultrasonic Disintegrator Measuring & Scientific Equipment Ltd England) and centrifuged at 1 000 × g for 15 minutes

Gel Filtration and Anion Exchange Chromatography The three erythrocyte protein fractions (A B and C) respectively were equilibrated with 0 01 M sodium phosphate buffer pH 7 35 by gel filtration on suitable Sephadex G 50 coarse grade columns (6 cm × 56 cm) with the buffer as the eluant The macromolecular fractions in the column effluents were transferred to DEAE Sephadex columns (3 cm × 12 cm) prepared for use as described by the manufacturers and equilibrated with the sodium phosphate buffer The columns were subsequently eluted with the sodium phosphate buffer containing 0 20 M NaCl When the optical density of the column effluent (OD 280 mμ) approached zero further elution was carried out with sodium phosphate buffer containing 0 40 M NaCl The macromolecular fraction of the second elution step were dialysed against distilled water for 48 hours lyophilized completely desalted by gel filtration on Sephadex G 50 columns (2 cm × 28 cm) with distilled water as the eluant and re lyophilized The weight of the final residue was recorded

Agar Gel Electrophoresis and Immunoelectrophoresis The equipment of LKB Produkter Sweden was employed For each protein fraction to be analysed electrophoresis on agar plates in triplicate was carried out One plate was used for developing precipitation arcs with antisera according to the micromethod of Scheidegger (9) The two other plates were fixed in a 4 per cent (w/v) solution of acetic acid and stained with Oil Red 0 (12) and benzidine (13) respectively

Vertical starch gel electrophorsesis was carried out in borate buffer pH 8 6 (0 3 M boric acid — 0 06 M NaOH) as described by Smithies (10) The equipment of O Hiller USA was employed The starch gels were stained with Amido-black (E Merck AG Germany) in 1 per cent (w/v) acetic acid and excess dye removed with a 7 per cent (w/v) aqueous solution of acetic acid In addition benzidine staining was also carried out

RESULTS

On anion exchange chromatography hemoglobin was almost completely eluted with 0 20 M NaCl Only tracer amounts could be detected in

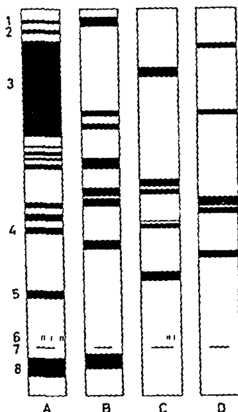


Fig 1 Drawing of vertical starch gel electrophoresis patterns (anode at the top)
A *Human plasma* (50 µl 1 tryptophanrich prealbumin 2 acidic alpha 1 glycoprotein 3 albumin 4 transferrin 5 alpha 2 macroglobulin 6 beta lipoprotein 7 site of application 8 gamma globulin
B Supernatant of freeze-destroyed erythrocytes
C Ghosts treated with desoxycholate
D Ghosts treated with ultrasound

the subsequent elution step (0 40 M NaCl) The dry weight of the material in this latter step was 12 mg/100 ml blood corresponding to fraction A 5 mg/100 ml corresponding to fraction B and 11 mg/100 ml blood corresponding to fraction C Material from these residues was dissolved in a 0 9 per cent (w/v) NaCl solution to a final concentration of 5 per cent (w/v) and subjected to agar gel electrophoresis

and vertical starch gel electrophoresis In the case of immunoelectrophoresis 2 5 per cent and 10 per cent (w/v) solutions were also investigated

Red Oil 0 stainable material could only be detected in the desoxycholate treated fraction (B) spreading out in a dense zone in agar gel electrophoresis from the whole of application to the position of albumin Immunoelectrophoresis revealed that this fraction did not contain alpha 1 alpha 2 or beta lipoprotein

In material corresponding to fraction A a single precipitation arc in the alpha 1 globulin position could be detected when horse immune serum against human serum was employed The position of this precipitation arc corresponded to that of alpha 2 macroglobulin Immunoelectrophoresis using specific antiserum against this latter protein however revealed that the precipitation arc did not represent alpha 2 macroglobulin

No more precipitation arcs could be detected in the material investigated by immunoelectrophoresis using the different antisera available

By vertical starch gel electrophoresis several protein zones were seen corresponding to each of the three protein fractions investigated (Fig 1) From the positions of the zones the presence of 10—14 different proteins is indicated spreading from the gamma G globulin position to that of tryptophanrich prealbumin

In order to insure that the urate binding alpha 1 — alpha 2 globulin had not been destroyed by either desoxycholate or ultrasound the following experiments were carried out Material from the plasma protein fraction containing this protein (1) was redissolved in physiological (0 9 per cent w/v) NaCl solution treated with desoxycholate and ultrasound respectively and subsequently separated by anion exchange chromatography as described under Methods When the final material was studied by immunoelectrophoresis employing the rabbit immune serum against the urate binding alpha 1 — alpha 2 globulin a typical precipitation arc developed in both cases

DISCUSSION

In the present work three different extraction methods were employed for isolating human erythrocyte proteins For the liberation of red cell membrane proteins butanol extraction has previously been used (7) In the present work the lipoprotein part of the erythrocyte membrane was

solubilized by desoxycholate in accordance with the method used by Korner (4) for solubilization of microsomal proteins Lipid staining of the material obtained indicated that lipids had been solubilized to a considerable degree

By the three different extraction methods in combination 10—14 non hemoglobin protein zones could be distinguished in vertical starch gel electrophoresis In previous reports 8—10 non hemoglobin erythrocyte proteins have beeen detected (2 11) The results obtained in the present work therefore seem quantitatively satisfactory as far as the material studied only represents one step of elution of erythrocyte proteins from DEAE Sephadex columns

None of the proteins detected could be identified as corresponding to the urate binding alpha 1 — alpha 2 globulin As it has been demonstrated that the procedures employed do not influence the behavior of this protein on DEAE Sephadex columns its electrophoretical mobility or its immunological properties it therefore seems rather unlikely that human erythrocytes contain the urate binding alpha 1 — alpha 2 globulin It cannot be ruled out however that the urate binding alpha 1 — alpha 2 globulin may be involved in the transport of urate from plasma to the erythrocytes by a reversible attachment to the red cell membrane parallel to what has been proposed for transferrin in iron metabolism (3) and lipoproteins in phospho lipid metabolism (8)

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metabolism (4) No reports of radioactive Urographin studies in rheumatoid arthritis are available however The method used in this study allows simultaneous recording of the decay of radioactivity in the joint and the increase of radioactivity in the blood and visual surveillance of the disappearance of the contrast substance from the joint

MATERIAL AND METHOD

Ten patients with classical RA (Fourth Department of Medicine Helsinki University Central Hospital and the Rheumatism Foundation Hospital Heinola) and nine patients with traumatic injury of the knee joint (First Department of Surgery Helsinki University Central Hospital Maria Hospital and the Orthopedic Hospital of the Invalid Foundation) were investigated The clinical data of the rheumatoid and traumatic groups are presented in table I

Ten ml of a 60 per cent solution of Urographin (Schering AG Berlin) mixed with 20 μC ^{125}I labelled Urographin (Radiochemical Centre Amersham) were injected by puncture of the joint cavity according to a method described earlier (7) Radiographs in four standard stereo-projections were taken during the period 3—9 minutes after the injection The absorption pattern was studied in lateral projection radiographs taken 10 25 40 55 90 120 180 minutes and 4 and 6 hours after the injection

The radioactivity was counted with a Wallac scaler model Sc 23 equipped with an analyser ratemeter AS—11B/ and a detector type SCDA—4 with a quartz and silica plane crystal size 1¾" x 2' (Turku Finland) The values for the radioactivity in the knee joint are means of three counts for 20 seconds with the detector directly over the patellar region at 2 10 20 30 45 60 90 120 180 minutes 4 6 and 24 hours after the injection The detector tube was shielded allowing impulses from a standard area 64 mm in diameter

For determination of the radioactivity in the circulation 2 ml blood was drawn from a cubital vein at 15 30 45 60 120 180 minutes 4 6 and 24 hours after intra articular injection of the isotope solution For counting the blood samples a detector SCDA—4 with 1¾" x 2 well crystal and a well size of 17 x 39 mm was used Continuous monitoring of the thyroid excluded the possibility that radioactivity was accumulating in the gland No increased activity could be detected after

TABLE I

The Clinical Data of the Patients in the Rheumatoid Arthritis (R) and Traumatic (T) Groups

Rheumatoid arthritis — R group							
Number of patient	Sex	Age	Duration of disease	Duration of knee joint involvement	Degree of knee joint inflammation	ESR	Rh F
1	f	18	2 months	1 month	3	110	seronegative
2	f	28	6 years	1 month	2	2	seropositive
3	f	37	10 years	3—4 months	3	65	seropositive
4	f	3[illegible]	10 months	6 months		63	seronegative
5	f	19	4 years	6 months	2	43	seronegative
6	f	35	7 years	1 year	2	35	seropositive
7	f	16	13 years	4 years	3	21	seronegative
8	f	20	9 years	5 years	3	106	seropositive
9	f	49	6 years	5 years	2	[illegible]	seropositive
10	f	56	8 years	8 years	3	1[illegible]0	seropositive

Controls — T group				
Number of patient	Sex	Age	Time after trauma	Hydrops
1	m	26	3 days	+
[illegible]	m	22	3 weeks	+
3	m	32	4 weeks	+
4	m	23	6 weeks	—
5	m	47	[illegible] months	—
6	m	46	[illegible] months	—
7	m	61	7 months	+
8	m	51	3 years	—
9	m	46	4 years	—

1 = Minor involvement (Tenderness + pain on use ± thickening + effusion ±)

2 = Moderate involvement (Heat + tenderness + or + + pain at rest ± pain on use + or + + thickening + effusion — + or + +)

3 = Severe involvement (Heat + tenderness + + pain at rest + or + + pain on use + or + + thickening + effusion + or + +) (according to Harris et al. (5))

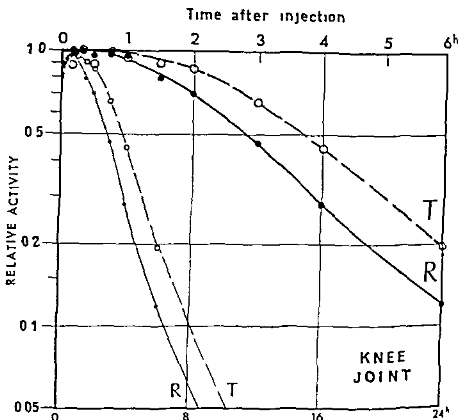


Fig 1 Mean logarithmic curves for the radioactivity over the knee joint in the rheumatic series (R) and the traumatic series (T) Heavy line = 0 to 6 hours thin line = 0 to 24 hours

twenty four hours Some individuals were pretreated with 5 per cent potassium iodine solution 20 drops three times daily prior to the experiment After the experiment no differences in thyroid activity could be shown between iodine treated and untreated persons

In order to obtain a representative picture of the two groups the variations in level between the activities observed in the individual patients were eliminated by the following procedure The means of the logarithms of all recorded counts were calculated The 24 hour counts over the knees did not differ from the background and were excluded For each set of values the additive correction was found to make the means of all subjects equal

The level of the curves constituted by the mean values obtained for

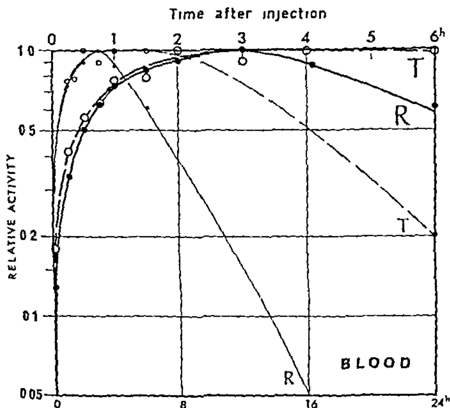


Fig 2 Mean logarithmic curves for the radioactivity in the blood in the rheumatic series (R) and the traumatic series (T) Heavy line = 0 to 6 hours thin line = 0 to 24 hours

different times of observation after the set correction was finally adjusted to make the maximum exactly equal to 1 0

The mean curves thus obtained represent the average behaviour of the subjects in terms of observed activity in per cent of the average maximum activity

RESULTS

The mean curves obtained by the above procedure for the activities observed at the knee joint have been plotted in fig 1 the heavy curves for the time from 0 to 6 hours (time scale at the top) for the rheumatic and traumatic patients (R and T respectively) in addition to which the

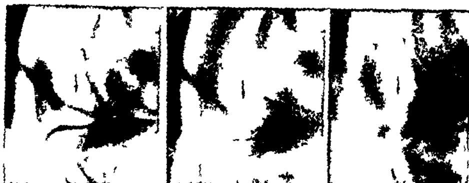


Fig 3 Case no R 9 The arthrograms were taken a) *10 minutes*, b) *60 minutes* and c) *90 minutes after injection of Urographin* Absorption of contrast *in the* articular cartilage and in the fibrocartilaginous tissue of the same m niscus can be *seen*

thin lines in the figure show the same curves in the interval from 0 to 24 hours (time scale at the bottom)

Fig 2 is a similar presentation of the more rapid changes of activity in the R series compared to the T series No statistically significant differences could be established between the individual plotted means referring to identical times after injection

In the R series the activity observed at the knee joint seems to reach maximum intensity (1 0) very soon within 10—15 minutes against about 30—45 minutes in the T series It also starts to fall earlier and at a faster rate in the R series Below are shown the times taken for the activity to decline to certain degrees

Fraction	Time R series	Time T series
1/2	2 85 hrs	3 70 hrs
1/5	4 75 hrs	5 95 hrs
1/10	6 5 hrs	8 25 hrs

The rate of decline of activity at the time when about half the maximum activity has disappeared is equivalent to about 1 45 hrs in the R series and 1 65 hrs in the T series

The maximum average count level at the knee joint in the R series prior to correction of the set level was 78 per cent of that in the T series



Fig. 5. Case no. R 8. Arthrogram after injection of 10 ml of 60 % Urographin. Rounded prominent portions and irregularities can be seen in the capsular outline. The menisci are uneven.

The curves relating to blood activity in fig. 2 reveal a virtually equal rise of the activity to the maximal point 1.0 about 3 hrs after injection. In the T series the average activity then remained at practically the maximum level up to about 8 hrs, while in the R series it began to fall immediately after 3 hrs. The times taken to reach various fractions of the maximum, read from the graphs, are:

Fraction	Time R series	Time T series
1/2	7 hrs	16 hrs
1/5	11 hrs	24 hrs
1/10	13.5 hrs	? hrs

In the R series from about 6 to 10 hrs the half life time would seem to be about 5 hours perhaps becoming shorter later on In the T series the half life time is about 7 hrs and 6 hrs in the 10—15 and 15—20 hr intervals respectively

The tendency for the disappearance of Urographin seems to be shorter in acutely inflamed rheumatoid joints than in long standing rheumatoid synovitis

The main route for disappearance of contrast is through the synovial membrane Clear evidence of the absorption of Urographin by the hyaline cartilage and fibrocartilaginous tissue of the menisci was obtained from the radiographs (Fig 3) In case number 5 with RA (Table I) the popliteal lymphatic vessels and lymphatic glands are visualized on the radiographs taken 10 25 and 40 minutes after injection (Fig 4) This observation shows that the lymphatics take part in the removal of synovial fluid components The Urographin solution disappeared from the lymph nodes within 45 min This reveals rapid lymphatic transportation

DISCUSSION

It can be stated that in an active rheumatoid joint the rate of removal of molecules of various sizes is enhanced in comparison with the control joints This has previously been demonstrated (1 5) with molecules of smaller and larger sizes than the Urographin used in this study The use of a labelled x ray contrast medium offers some advantages because tracing can be combined with visualization of the joint This is of importance to establish the success of the injection and to obtain diagnostic information at the same time

Because no danger is attached to the method and the Urographin does do any damage (2 3) it can be recommended for routine purposes s an indicator of the dynamics of a joint as well as for arthrographic follow up studies In one case of RA there was clear arthrographic idence of lymphatic elimination of contrast In all the other knee joints ffusion into the hyaline cartilage and fibrocartilage was obvious A of the pathways of elimination from a joint raises many interesting uestions We intend to continue our investigation by comparing the limination into the blood system and the lymphatic system using a fistula the thoracic duct (8) In addition the use of radioactive contrast es of different molecular sizes may yield valuable informati

concerning the best method of drug binding in intra articular treatment The time of disappearance of a drug can then be chosen

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REVERSIBLE ACUTE RENAL FAILURE COMPLICATING RHEUMATOID ARTHRITIS

By

OTTO WEGELIUS and MATTI KLOCKARS

Summary Although morphological and functional kidney lesions are well known to occur in rheumatoid arthritis (RA) there appear to be no previous reports on reversible acute renal failure That interstitial nephritis plays a part in acute renal failure is clinically documented but the pathophysiology is obscure The role of raised interstitial pressure has often been discussed but convincing evidence is still lacking

Two patients with classical RA complicated by reversible oliguria and impaired kidney function are presented Renal biopsies revealed no glomerular tubular or amyloid lesions The only light microscopic finding was greatly increased interstitial edema which stained metachromatically with toluidine blue and was hyaluronidase sensitive

It is supposed that the abnormal concentration of interstitial hyaluronic acid and its high water binding capacity leads to an increase of interstitial pressure, which is followed by renal insufficiency The mucinous edema of the kidneys is assumed to be an acute reaction of the pathological connective tissue in general

The most common causes of acute renal failure are accepted to be renal

Aided by grants from the S gr d Ju elius Foundation Helsi ki, Finland

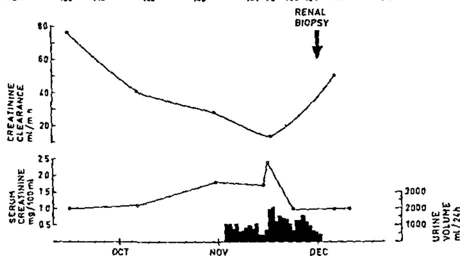
ischemia as a result of shock or hemorrhage renal vascular lesions or severe glomerular lesions and direct tubular cell damage by toxic agents The pathogenetic events in acute renal failure are not understood especially when no irreversible destruction of renal tissue is found According to Zollinger (12) the clinico pathological entity acute interstitial nephritis may also lead to renal failure and exitus due to uremia The pathogenesis of interstitial nephritis is poorly understood Theories have been put forward in favor of immune inflammation an allergic process shock or collapse and ischemia (7 12)

In RA renal involvement is well documented Clinical manifestations of renal disease appear in 10—30 % of the patients The morphological features consist of focal glomerulitis glomerulonephritis vascular lesions and renal amyloidosis (2 3 8) The existence of interstitial nephritis has been reported and has been attributed to increased phenacetin consumption On the other hand the interstitial connective tissue of the kidneys in RA may react with acute morphological and functional changes as a sign of the disease itself The two patients reported here exhibited signs suggestive of increased disease dependent connective tissue susceptibility resulting in mucinous edema of the renal medulla and oliguria

CASE REPORTS

Case No 1 Woman of 43 Her rheumatoid disease began at the age of 31 after pharyngitis its onset was typical with mild joint symptoms pain and swelling The disease has progressed into a classical case of severe RA affecting all synovial joints Treatment has consisted of aureothiomalate chloroquine phosphate phenylbutazone salazopyrine analgetics and glucocorticoids but no phenacetin treatment During the past year she has been on dexamethasone treatment and developed steroid diabetes

Before admission to hospital severe progression of the joint symptoms was noted On admission hypercortisonism was evident the blood glucose being 200 mg/100 ml and the ESR over 100 mm/h Serological tests for RA were negative electrophoretically the serum alfa 2 and gamma globulins were increased Kidney function serum creatinine 1 0 mg/100 ml creatinine clearance 72 ml/min Urine volumes and urine sediment were normal The development and regression of the kidney disease are shown in figure 1



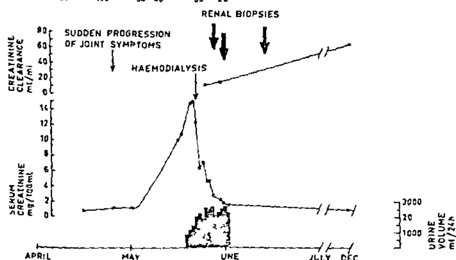


Fig 1 The serum creatinine values creatinine clearances and urinary volumes in two patients with exacerbation of RA and renal failure

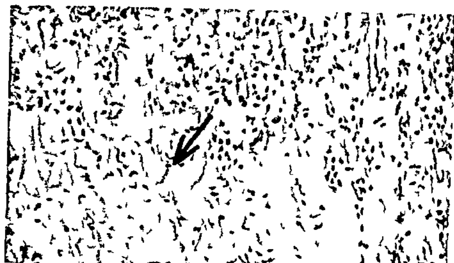


Fig 2 Patient number 2 biopsy number 1 Pronounced interstitial edema in the medulla of the kidney The area below and around the arrow showed strongly positive histochemical reactions for acid mucopolysaccharides 175 × Hematoxylin eosin

Case No 2 Woman of 60 She has had intermittent unspecific joint symptoms for ten years In February 1963 she caught a common cold with fever cough and small vesicles in her mouth During this episode joint swelling stiffness and pains were increasingly observed She had received no treatment before hospitalization

On admission in April on account of her joint disease the ESR was 101 mm/h and the blood exhibited eosinophilia ad 990/mm³ X ray of the chest showed spotty infiltrations Bronchoscopy and bronchography were normal No sign of kidney disease was noted

After a sudden progression of the joint symptoms ten days later the Waaler Rose and latex fixation tests became positive Prednisolone 15 mg/day gave subjective relief and objective disappearance of the lung infiltrations for a few days After two weeks stay in hospital she developed acute abdominal pains vomiting and later icterus Her kidney function tests showed rapid and progressive impairment The serum creatinine values rose to 10 mg/100 ml and the serum potassium level to 6 45 mEq She had no symptoms from the urinary tract The daily urine volumes decreased The renal disease progressed for five days the serum creatinine rose to 15 mg/100 ml and she was admitted to a renal ward for hemodialysis Improvement after a single treatment was

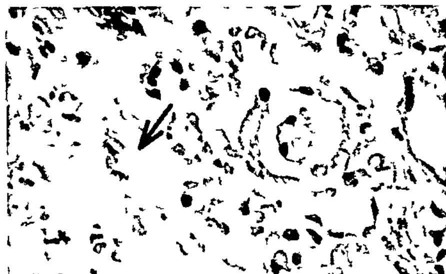


Fig 3 Patient number ? biopsy number 1 The edematous interstitium surrounding non functioning tubules 750 × Hematoxylin-eosin

rapid and the renal function tests normalized within a week (Fig 1) Renal biopsies were performed three times during the acute course and the histological findings are shown in figures 2—4

HISTOLOGICAL FINDINGS

During the acute course of the kidney disease renal biopsies were taken (B Kuhlbäck see fig 1) The specimens were fixed in neutral formalin embedded in paraffin wax sectioned at 4 μ and stained with periodic acid Schiff hematoxylin eosin van Gieson Congo red 1 per *cent toluidine blue and Alcian blue Some sections were treated with* hyaluronidase (Hyason Organon Oss Holland) and then stained with Alcian blue

Patient no 1

In a representative biopsy specimen only normal glomerular structures normal tubular cells and no deposits in the tubules were seen Only a few atrophic tubules could be detected The major changes were in the interstitial tissue which showed marked edema and only a f

reported may be due to such congestion, to increased permeability of the vascular bed and/or to direct pathological stimulation of the mesenchymal cells. A similar connective tissue reaction with accumulation of ground substance components has previously been observed in the heart in scleroderma (11).

In the present cases the toxic effect of some drug may be responsible for the interstitial reaction of the kidneys. The fact that similar drugs had been used earlier and have been used later argues against such an explanation, but the possibility of a drug induced nephritis cannot be ruled out.

Experimental data and clinical experiences of interstitial reactions due to massive glomerular filtration and tubular reabsorption of small molecular substances are on record. Recent experimental findings (10) show that even native tissue homogenate per se causes interstitial nephritis around the proximal convoluted tubules. Prior to the oliguric phase the two patients with rheumatoid disease showed an exacerbation with severe progression of the joint symptoms. Tissue components liberated in increased amounts into the circulation may be speculated to act as nephritis inducing substances by the same mechanism.

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The Rheumatological Association of Sweden held its Annual Meeting in Stockholm on November 27 1969 At the meeting the following papers were presented

Total Complement (C) Concentration and Conversion of the Third Component (C3) of C in Synovial Fluid from Arthritic Patients

By H Hedberg B Lundh and A B Laurell (Lund)

To elucidate the phenomenon of a suppression of C in synovial fluid the concentration of C3 and the occurrence of a C3 conversion in synovial fluid were studied

The C3 concentration in synovial fluid immunochemically determined was lowest in cases of systemic lupus erythematosus (SLE) and SLE like syndromes and in cases of sero positive rheumatoid arthritis (RA) In the whole series of synovial fluids including fluids from patients with ankylosing spondylitis Reiter s disease and psoriatic arthropathy the C3 concentration proved to be correlated to the total C

Under certain conditions the (native) C3 (the beta 1C globulin) can be converted into various conversion products For example upon exposure to immune complexes an electrophoretically somewhat faster conversion product appears (Muller Eberhard & Nilsson *J exp Med 111* 217 *1960*) *Instead of ordinary immuno*-electrophoresis which yielded discouraging results in our hands we have used antigen antibody crossed electrophoresis (Laurell Anal Biochem *10* 358 1965) to demonstrate C3 conversion By means of this technique significant C3 conversion was demonstrable in certain synovial fluids notably in those with low levels of C and/or C3 Fluids showing the most pronounced C3 conversion (>30 % planimetrically determined) all had extremely low C values and they were all derived from RA patients with positive tests for rheumatoid factors There was no detectable C3 conversion in (EDTA) plasma not even in plasma from patients with active or moderately active SLE

The results seem to support the view of an in vivo activation of the C system in the rheumatoid joint

Impaired Phagocytosis by Peripheral Blood Granulocytes in SLE

By L Brandt and H Hedberg (Lund)

Using heat killed yeast cells for the assay of the phagocytic activity (Brandt L Scand J Haemat Suppl 2 1967) phagocytosis was studied in 12 normals 7 SLE patients and 7 patients with rheumatoid arthritis (RA) Comparable daily doses of prednisolone were given in the two groups of patients Phagocytosis proved to be indistinguishable in the normal group and in the RA group and almost identical with that previously found in RA patients treated with phenylbutazone In the SLE patients the phagocytic activity was significantly lower than that found in the 3 control groups mentioned

Phagocytosis was not reduced when normal granulocytes were suspended in SLE plasma nor did the phagocytic activity of SLE granulocytes increase in normal plasma Leucocyte agglutinins could not be demonstrated in sera from the SLE patients The lowe t phagocytic activities found in the SLI group tended to be associated with neutropenia which may be a drawback in the defence against infections

Urinary Excretion of Free Immunoglobulin Light Chains in RA and Other Connective Tissue Diseases

By Folke D Lindstrom (Department of Rheumatology University Hospital / Lund)

Free light chains are normally excreted in urine They are regarded as a spill over product from normal immunoglobulin synthesis

Urine concentrates from 32 patients (20 RA 4 juv RA 3 SLE 2 systemic sclerosis and 3 various conn tissue disease) were examined with agar gel electrophoresis immuno electrophoresis and immunoglobulin quantitation using *the Oudin tube method*

Increased amounts of free light chains were found in urine from the patient group (mean 39 6 mg 24 h) as compared to the control group of 20 individuals *(mean 9 4 mg/24 h)* The difference was statistically significant

The immunoglobulin excreted generally showed no evidence of increased homogeneity on agar gel electrophoresis In one of the patients with systemic sclerosis however a Bence Jones like protein was excreted in the urine appearing as homogeneous band on electrophoresis and consisting of K type light chains exclusively

The increased urinary excretion of free light chain is thought to indicate an increased immunoglobulin synthesis in the patient group The occurrence of a

ence Jones like protein in the urine from a patient with systemic sclerosis has ot been described earlier The significance of this finding is obscure

tudies of the Intestinal Conditioning Factors for Clostridium Perfringens in :A and Other Collagen Diseases

By Borje Olhagen Ingmar Mansson and Gunnar Havermark (Karolinska Hospital and Royal Veterinary College Stockholm)

A special type of Clostridium perfringens belonging to group A has been ecovered from the intestinal contents of patients with RA and SLE in two thirds f the cases Also patients with other forms of chronic arthritis such as psoriatic rthropathy ankylosing spondylitis and seronegative arthritis showed this bnormality The isolated strains differ from normally occurring clostridia with espect to lecithinase activity thermoresistance and certain biochemical properties Circulating and cell bound antibodies against C. perfringens alpha toxin was lemonstrated in high frequency in RA

By intubation of the small intestine it was detected that the abnormal clostridia ippear at a level as high as the duodeno-jejunal flexure — The findings are regarded as evidence of a change in the milieu interieur of the host notably the ntestinal tract An analysis of various non rheumatic conditions with probably solated functional changes in the digestive glands shows that chronic sialoadenitis with severe sialopenia (Sjogrens syndrome without arthritis) does not result in abnormal C perfringens intestinal flora nor does obstruction of bile flow or exocrine pancreatic insufficiency (chronic pancreatitis) The pH of the gastric juice per se is not of importance for the occurrence of intestinal clostridia in arthritis However patients with histamine refractory achylia often display high counts of C perfringens in feces The strains isolated in achylia belong to a type of C. perfringens group A other than the arthritic strains as regards thermo resistance and biochemical properties Leakage of plasma proteins or blood losses into the gastrointestinal channel also result in a definite increase of the number of fecal clostridia the strains isolated are of the achylia type however

Various dietary regimens such as chemical diet (amino acids essential fatty acids glucose and vitamins) vegetarian food and gluten free diet have been tried without any significant effects on the abnormal flora As to the effects of antibiotics on the fecal clostridia it was noted that ordinary penicillin preparations do not influence the abnormal flora whereas the penicillinase stabile cloxacillin seems to eradicate the clostridia in the stools The same experience has been with tetracyclines However in part of the cases the abnormal bacteria reappeared in the stools after one to three months so evidently a development of resistance to tetracycline is possible

Studies on the Diet Induced Polyarthritis in Pigs

By I Månsson N E Bjorklund R Norberg and B Olhagen (Royal Veterinary College and Karolinska Hospital Stockholm)

After a diet based on cereals but also containing fish meal 20 % had been fed ad lib to 8 weeks old pigs a significant and persistent rise in the number of intestinal Clostridium perfringens was obtained in 1—2 weeks The pigs developed signs of polyarthritis clinically shown by swollen joints and a typical stilted and stiff gait Elevated ESR values raised alpha antitoxin titers in serum and hypergammaglobulinemia were also noted

An increased amount of joint fluid could be demonstrated In some pigs it seemed to be normal but in others it was turbid and contained granulocytes (80 %) lymphocytes (10 %) and monocytes (10 %) Stratum synoviale was edematously thickened and histologically a highly vascularized granulation tissue with fibroblasts angioblasts macrophages and other reticular cell elements could be seen Suggestions of necrosis with concentric arrangement of surrounding mesenchymal tissue and hyperplasia of the synovial membrane were observed Villi were hypertrophied and accumulations of mononuclear cells were found Pannus formation was also noticed

In pigs in which the arthritis had been persistent for several weeks the attachments of the joint capsule were inflamed and a profuse fibroblastic and osteoblastic proliferation was found A broadening of the epiphyseal cartilage zone was seen and erosions in the articular cartilage Examinations for the presence of bacteria and mycoplasmas in the synovial fluid were negative

Intra Articular Radiation Therapy in RA

By Wilhelm D son Aschan Anders Movin and Lennart Sundbom (Eskilstuna General Hospital)

Surgical synovectomy has proved to have a particularly good effect in early cases of RA while in later stages of the disease (Steinbrocker s stages II and III) it mainly seems to give alleviation of pain for a short period In the latter cases another common effect seems to be limitation of movement Intra articular injections of steroids and alkylizing substances have up to the present given only transient effects

There are still only a few reports on the effect of intra articular injection of radioactive isotopes The method has been applied at the Eskilstuna General Hospital since 1967 the tracers used being radioactive gold (Au^{198}) and radioactive phosphorus (P^{32}) The radiation doses given have varied but they usually correspond to those recommended by Barbara Ansell et al Practically all joints in

the extremities have been treated Nausea and headache after the injection disappearing within about 24 hours have been noted in one case and local side effects such as pain and increased swelling in the irradiated joint in five cases To find the right therapeutic dose for each individual joint proved to be difficult perhaps we do not vary the dose enough Neither do we know whether the full amount of isotope should be injected all in one dose or whether it should be given in several doses as in roentgen therapy

Several weeks or a couple of months in isolated cases even up to six months usually elapsed before the patient experienced any improvement in the irradiated joint As in other forms of treatment including surgery the duration of the beneficial effect varied in those cases that responded favorably Here also however we are faced with the difficulty of assessing how much of the effect may have been due to the concurrent general treatment and how much to spontaneous regression of the disease

No cases of early RA have been treated Our material consists without exception of cases in Steinbrocker s stage II or stage III Because of the possible risk of damage to the gonads wom n of fertile age have not been treated The age distribu tion in the material is consequently slightly askew In all cases the injection was given immediately after an arthrographic examination and via the same needle as had produced a perfect arthrogram

Radioactive gold was given to 92 patients 36 of whom were men and 56 women *In 81 cases the diagnosis was RA and in 11 arthrosis deformans A total of 170* joints were treated in the RA cases and 13 joints in the arthrosis deformans cases

The observation time varied from 10 to 32 months The cases with an observation time of one year or more are however of special interest in the present context and in these the following effects were noted *Spontaneous pain* Nearly half the patients had no spontaneous pain at all 21 % had less pain than before and one third still had pain of exactly the same severity as before the injection *Pain on movement* One third of the patients had no pain at all one third had less pain and one third had pain of the same severity as before *Range of move ment* 48 % of the patients had a wider range (10% or more) while the others had the same range as before In no case was a reduced range noted Among the arthrosis deformans patients six showed no improvement with respect to pain on movement and initial stiffness five were slightly improved and in two only slight discomfort persisted

Radioactive phosphorus (P^{32}) has up to the present been injected into an increasing number of joints in the limbs in the beginning only knee joints were treated *and it is only among these that a follow up material is available for* report This series consists of 35 knee joints and the observation time was 6—18

months with the average at 10 months All patients had had spontaneous pain pain on movement and weight bearing and swelling before the treatment Results *Spontaneous pain* Unchanged in one case better in nine cases and symptom free in 25 cases Corresponding figures were 3 12 and 20 for *pain on movement* 6 11 and 18 for *pain on weight bearing* and 2 12 and 21 for *swelling* With P^{32} the effects of the treatment were noted 1—26 weeks (average 8 weeks) after the injection In eight patients the effect was transient and the pain returned after 1—9 months In two patients the treatment had no effect at all

Thus the preliminary impression is that P^{32} has a more favorable effect than Au^{198}

The particle sizes in the radioactive colloidal gold and phosphorus compounds were 50—200 A and 300 A respectively Au^{198} emits both gamma and beta radiation while P^{32} emits only beta radiation In order to permit external recording of the radioactivity in the joint under treatment the regional lymph nodes and the liver both when Au^{198} and P^{32} were given a chromium phosphate also containing radioactive chromium was used These recordings were made 24 hours one week one month and in certain cases three months after the injection The greater part of the colloidal substance is absorbed in the synovial tissue Varying amounts however are transported via the lymphatic system mainly to the regional lymph nodes but also to other lymph nodes and to the liver as well as in small amounts to other organs The external recordings of the radioactivity showed that the radiation dose in the liver was less than 10 rads except in one case where the dose was estimated to be of the magnitude of 50 rads The radiation dose in the lymph nodes was often relatively low but in two cases it was estimated to be 7 000 rads and in one case 15 000 rads The dose in the irradiated joint was hard to calculate mainly because of difficulties in determining the area of the synovial tissue and the distribution of the colloidal substance in the tissue For 5 mCi of gold and 1 mCi of phosphorus the maximum dose in a knee joint of normal size will be about 18 000 rads if all the activity is distributed evenly over the synovial tissue The dose decayed rapidly for radioactive gold and was calculated to be 5 000 rads at the 0 4 mm depth in the tissue For phosphorus the corresponding depth was 1 mm At a depth of 2 mm values of about 700 and 2 000 rads were obtained for gold and phosphorus respectively Because of the differences in the half lives for these two radionuclides the dose rates will be different For the first seven days the accumulated dose for phosphorus was approximately only a third of the dose for gold After 15 days only half the total dose had been obtained in the patients treated with phosphorus while in those given radioactive gold practically the entire dose had been obtained

Knee Surgery in RA

By Håkan Brattström (Lund)

A survey is given of the possibilities of today's knee surgery in RA. Preventive as well as reconstructive surgery becomes more and more usual.

A Study of Organization and Efficiency of Ambulant Rehabilitation in Rheumatology

By M. Brattström and K. Berglund (Department of Rheumatology, University Hospital, Lund)

The object of the test was to build an organisation that on an ambulant basis could solve as much as possible of the medical, functional and social problems of patients invalidated by rheumatic diseases. This included the development of methods for controlling the efficiency of the service. A physician, a physiotherapist, an occupational therapist and a social worker cooperated closely. For each of these the measures undertaken were recorded. Appraisal of the patients resulted in formulation of differentiated objectives.

Results of measures and the extent to which each objective had been attained were estimated at 3, 6 and 9—12 months after the beginning of the treatment. All these data were recorded on punch cards and an analysis of the results, a "production control", was made with the aid of a computer.

Most of the 285 patients were more or less incapacitated (177 in functional class's III and IV according to the criteria of the American Rheumatism Association). Some encouraging results can be mentioned. Measurable improvement of practical value for the patient was obtained in all groups concerning range of motion and strength of legs (best in functional classes II and III). The majority of patients in functional classes II and III could be learnt to dress with technical aids and adequate training. The functional capacity of patients in functional class IV could be improved by technical aids as lifts, wheel chairs etc. following a thorough evaluation of their needs.

In many instances the measures did not lead to attainment of the objectives. We feel that the efficiency of the service will gradually increase by this production control. It will initiate improvement of methods and elimination of meaningless measures.

Patient's and Doctor's Evaluation of Symptoms in RA

By Erik Allander (Dept. of Social Medicine, Karolinska Hospital, Stockholm)

To obtain a *non selected population of persons suffering from different stages* of RA, random samples were drawn of certain age groups in the population of

Stockholm Four quinquennial age groups 31—74 years old altogether 15 768 persons were investigated by questionnaire and a representative sample was *examined physically* Out of 293 persons found to have RA 80 % fulfilled ≥ 2 criteria for RA (New York 1966) X ray of hands and feet and serology were applied Several social and medical data were collected The severity of subjective joint troubles was graded by the rheumatics on a 6 point scale

The mean value for the grade of subjective troubles did not differ between age groups although the number of painful and/or tender joints was significantly higher in the oldest age group Generally subjective joint troubles correlated better with pain than with deformities or disability In Steinbrocker s functional class II all grades of subjective joint troubles were represented Although some correlation was noted between grade of subjective troubles and medical stage and manifestations of disease there were great inter individual variations in the degree of this correlation

There was no statistical difference between proportions of nervous persons in groups with slight vs severe subjective troubles Nor was any difference noted between the sexes with respect to grade of subjective joint troubles No statistical difference was noted between the number of criteria for RA and subjective grading of joint troubles

It was concluded that the difference in the evaluation of the severity and in the grading of the disease between persons with RA and a doctor is substantial and shows wide individual variations

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THE INTESTINE AND RHEUMATISM

By

BORJE OLHAGEN

Arthritis following certain Salmonella infections, dysentery, and non specific enterocolitis exemplifies the fact that intestinal infections can elicit rheumatic reactions. Recent observations indicate that in the last mentioned group specific microbes, such as Clostridium perfringens and Yersinia enterocolitica may be involved References are made to the findings of an abnormal intestinal flora, notably C. perfringens in patients with chronic arthritis and of circulating and cell bound antibodies to C perfringens alphatoxin as evidence of an immunologic response to this microbe in rheumatoid disease

The association between joint disease and intestinal disorders was scientifically documented as early as 1672 by Sydenham who described arthritis in dysentery With the advent of bacteriology the enterogenesis of a joint inflammation could sometimes be proved by the growth of pathogenic intestinal bacteria in joint exudate in for instance typhoid fever and brucellosis After the introduction of antibiotics the rare septic arthritis has virtually disappeared There remain however a variety of aseptic arthritides postinfectious joint reactions As regards intestinal infections three situations have long been known namely salmonellosis dysentery and non specific enteritis As for the salmonellosis it seems to be preferably a mild paratyphoid infection Salmonella typhimurium which can be accompanied by arthritis Accumulations of such cases have been reported both from Sweden and from Finland (4 31) being benign synovitis involving mostly large joints and of self limiting character

The most striking feature of the post dysenteric arthritis is of course *that it is often accompanied by symptoms referable to the mucous membrane of the urethra and the conjunctiva* the clinical picture which in 1916 was described simultaneously by Reiter (25) and Fiessinger & Leroy (7) In the discussion on the etiology of the Reiter Fiessinger Leroy syndrome the intestinal pathogenesis has usually been obscured by *the venereal aspect, possibly because of incomplete history taking* Fairly often it will be found that a patient with non specific urethritis has had an episode of diarrhea 2 or 3 weeks before the onset of the disease Shortly after Reiter s paper had appeared German workers had realized that non specific enteritis can also be *accompanied by acute arthritis* the so called polyarthritis enteritica (26) Subsequently Russian and French clinicians, in particular have emphasized this etiology At the time when France had armies in North Africa epidemics of non specific enteritis with accompanying polyarthritis and fairly often mucosal symptoms of Reiter type were observed in some seasons especially in August—September (15 24) Recently new aspects concerning a more specific etiology of this common enterocolitis have been brought forward Olhagen & Mansson (22) have observed that in acute arthritis *after non specific diarrheal disease entero arthritis a high frequency* of abnormal anaerobic flora could be demonstrated in intestinal contents namely a special type of Clostridium perfringens which belongs to group A but which in several important respects deviates from orthodox strains (see the following) Antibodies in serum against *alpha toxin* from these bacteria have also been found — Finnish workers have quite recently found a correlation between another intestinal microbe and arthritis namely positive agglutination reaction to Yersinia enterocolitica in *patients with postenteritic arthritis* (29)

It has thus been established that acute intestinal infections can be accompanied by acute self limiting joint reactions but it is also well known that joint symptoms often appear in chronic intestinal *disorders*, especially in ulcerative colitis but also in regional enteritis (Crohn s disease) Reports on the frequency of *joint symptoms in these diseases* vary between 15 and 25 % of the ulcerative colitis cases (17 33 35) and between 5 and 15 % of regional enteritis cases (1 8) The major part of these joint affections is *designated colitic arthritis*, which is a rapidly transient but often recurrent synovitis, notably of knee and ankle joints Prospective studies have however shown a relatively high frequency of sacroiliitis (18—20 %) usually clinically *silent but verifi*

able by x ray (8 34) In a small number of cases a clinical picture develops resembling that of ankylosing spondylitis peripheral joint changes in the form of erosive arthritis involving small joints are seen more seldom and are not as symmetrical as in rheumatoid arthritis and the sheep cell test is negative (17 33) The part played by the intestinal disease in the causation of the joint symptoms is illustrated by the fact that total colectomy results in the disappearance of the colitic arthritis symptoms (8 33)

The most striking example of how a clinically silent bacterial invasion of the intestine gives rise to symptoms referable to the joints is Whipple s disease The joint manifestations can precede the intestinal symptoms by years and years The clinical picture varies and resembles that of joint disease in ulcerative colitis (11) The characteristic PAS positive inclusions in the mucosa have by electron microscopy been found to be of a structure typical of bacterial cells but opinions differ as to the nature of these microbes anaerobic Coryne bacteria Brucella like bacteria Haemophilus Bacteroides (2 6 12 15) That the bacteria play a part is highly probable since treatment with tetracyclines possibly in com bination with cortisone relieves the patient of the joint symptoms The question of what is the primary defect in Whipple s disease has not yet been answered A recent suggestion is that it might be a disturbance in the cellular defence as a distinct lack of plasma-cells and lymphocytes in the submucosa has been observed in acute phases of the disease (16)

The point is whether intestinal microbes would have pathogenic implications in other rheumatic diseases Mansson and Olhagen (18 22 23) have drawn attention to a profound disturbance in the anaerobe intestinal flora in rheumatoid arthritis (RA) and other collagen diseases As in acute entero arthritis the fecal cultures from such patients show not only a significant increase in the number of Clostridium perfringens from ordinarily less than 100 to over 100 000 bacteria per g of feces but also that this flora has usually undergone a qualitative change The isolated strains are distinguished by a potent alpha toxin production i e high lecithinase activity moreover they differ in some important respects from ordinary strains of C perfringens in that they have a marked proteolytic ability and are extremely heat sensitive and their bio-chemical properties are also different Such bacteria do not seem to have been described earlier In RA and systemic lupus erythematosus (SLE) an abnormal flora was noted in two thirds of the cases in psoriatic arthropathy ankylosing spondylitis and chronic seronegative polyar

thritis about 60 % of the patients showed this abnormality The abnormal fecal flora was more rarely seen in Reiter s syndrome only exceptionally in post tonsillitic arthritis of streptococcal origin and never in post gonorrheal arthritis 0 9 % of the controls (1 out of 108) had the abnormal flora A tendency to higher frequency of abnormal C perfringens flora was noted in RA patients in whom the disease was highly active In patients with treatment induced remission the abnormal flora decreased to the range of normal Both circulating and cell bound antibodies to an alpha toxin preparation from C perfringens were found in high frequencies Histological examination of biopsy specimens from positive intracutaneous tests (papules) showed changes corresponding to a delayed hypersensitivity reaction of tuberculin type This hyperreactivity contrasts with the dermal hyporeactivity to other bacterial antigens such as tuberculin streptokinase and Brucella which earlier investigators have demonstrated in RA and in SLE (3 10 32)

The appearance of a new type of C perfringens in the intestine in rheumatoid disease is not the result of nosocomial infection Nor are there any such simple explanations as constipation or ingestion of aspirin or other drugs Olhagen & Mansson (23) look upon the findings as evidence for a change in the internal milieu of the intestinal tract Studies of various non rheumatic conditions with probably isolated functional changes in the digestive glands showed that for instance complete or almost complete absence of parotid saliva bile or pancreatic juice (chronic sialoadenitis obstructive jaundice and pancreatic achylia) does not seem to induce an abnormal clostridial flora On the other hand it was found that patients with histamine refractory achylia fairly often display increased numbers of C perfringens in their stools — which in fact was noted as early as the beginning of the 20th century (9) The strains that were isolated in achylia differ however in several respects from the arthritis strains for instance in their thermo-resistance The gastric acidity per se does not seem to play any decisive role in the occurrence of the abnormal C perfringens flora in RA and other forms of chronic arthritis for the frequency of the abnormal C perfringens was not higher in Diagnex negative than in Diagnex positive patients By intubation of the small intestine it was found that C perfringens appears even at the boundary between the duodenum and the jejunum in patients with RA but not in healthy persons It is evident that favourable growth conditions for C perfringens exist at this level in the rheumatic patients

The idea that RA might be of enteric etiology is of old date In the 1910s—1920s a popular concept among some investigators was that intestinal autointoxication originating from the upper parts of the colon would be the cause of RA These ideas led to for instance, drastic attempts at treating RA by colectomy which were then abandoned (30) Shatin (27 28) advocates that RA is a disease of intestinal etiology According to this hypothesis excessive consumption of gluten containing food particularly rye and wheat products would lead to malabsorption with secondary amino acid deficiency Shatin also reported good therapeutic results with a gluten free diet in RA Others have not been able to verify this (5 23)

Although it is improbable that the diet plays a primary role in the appearance of the abnormal C perfringens flora in RA and other inflammatory joint diseases it is of interest that Mansson et al have been able to induce arthritis in young pigs by feeding to them a diet rich in protein (19 20) The arthritis appears after only 8 to 10 days on the diet and affects mainly peripheral small joints bilaterally and symmetrically The abnormal Clostridium flora appears in the intestine of the pigs before the development of arthritis and is a prerequisite of it Moreover the arthritis is accompanied by an elevated erythrocyte sedimentation rate hypergammaglobulinemia and raised levels of alpha antitoxin in serum It seems probable that immunological mechanisms are responsible for the development of the arthritis It is also remarkable that the strains of C perfringens are almost identical with the strains which have been isolated in patients with RA and that this type of bacteria has so far been found almost exclusively in connection with inflammatory connective tissue diseases

In the experimental pig arthritis the diet is the primary factor in man it seems reasonable that a hitherto not elucidated disturbance in the digestive tract would result in altered growth conditions for this type of C perfringens It is of course possible that this supposed internal change in the intestinal canal might be the *result* of the rheumatoid disease through alteration in the function of the digestive glands or the mucosal secretions of something else Arguments for such an explanation are the finding of this flora in all forms of chronic arthritis and the fact that the higher frequency of abnormal clostridia is found in the most active stages of the disease On the other hand it is notable that the flora can be found also in the very early stages of RA in which only a few finger joints are affected and the ESR is still normal

A question for consideration later on is that of the relationship between this specific intestinal flora and the joints Indubitably C perfringens affords an immunological response in the host It is of special interest that cell bound reactivity to alpha toxin is so manifest considering that lymph node cells from animals with adjuvant arthritis can induce joint disease in healthy, immunologically tolerant animals of the same species

For those who insist upon separate etiological factors in for instance RA SLE psoriatic arthropathy the finding of an abnormal C perfringens flora in the intestine and the immunological reactions that accompany this phenomenon in most of the chronic inflammatory connective tissue diseases is confusing and hardly interesting as the basis for a discussion of the etiology question But the problem can be approached from other viewpoints Firstly there exists between the diseases in question a notable overlapping both clinically and serologically sometimes of such an extent that a differential diagnosis is impossible for instance between RA and SLE or between SLE and systemic sclerosis Secondly there are examples illustrating that particular clinical entities may have different pathogenesis and probably also different etiology The pharmacologically induced SLE syndromes are such an example Ankylosing spondylitis may appear as a rheumatic manifestation in at least five different diseases namely chronic prostato vesiculitis ulcerative colitis regional enteritis Whipple s disease and psoriasis Dermatomyositis may be induced by malignant tumour but can also appear without such an initiation There are cases of malignant lung tumour or pulmonary tuberculosis with concomitant RA in which the joint disease disappears after surgical treatment of the thoracic disorder (11 21) As long as the etiology is unknown it would perhaps be prudent to speak about clinical syndromes rather than about separate diseases The role of the intestinal clostridia in these rheumatic syndromes remains to be made clear but a gastro-enterological approach to the inflammatory connective tissue diseases seems a promising avenue of future research

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SJÖGREN'S SYNDROME ASSOCIATED WITH VITAMIN B_{12} DEFICIENCY

By

OTTO WEGELIUS FREJ FYHRQUIST and PER LENNART ADNER

Summary Three patients showing Sjögren's syndrome in association with macrocytic anemia due to vitamin B_{12} deficiency are described Antibodies to parietal cells, intrinsic factor and salivary protein were investigated, positive test results were obtained in different combinations Addisonian pernicious anemia as well as vitamin B_{12}-malabsorption because of anti intrinsic factor antibodies in gastric juice and/or malabsorption due to changes in the intestinal wall induced by Sjögren's syndrome are possible etiological factors A case of Sjogren's syndrome associated with positive anti parietal cell antibodies and histamine refractory achylia is also reported The concurrence of these two diseases known to be associated with autoimmune responses suggests the possibility of similar pathogenesis

In Sjogren's syndrome a chronic inflammatory process in the lacrimal and salivary glands due to secretory insufficiency of these organs leads to secondary changes in the cornea and conjunctiva and the mucous membrane of the mouth In over half the cases this syndrome develops during the course of rheumatoid arthritis or some other systemic connective tissue disease Initially the remaining patients exhibit their sicca syndrome as an independent disease Owing to the frequent finding of autoantibodies especially to salivary duct cells (2) this syndrome belongs to the group of etiologically unsolved diseases with autoimmune response (6)

Pernicious anemia develops on the basis of atrophic gastritis with

vitamin B_{12} malabsorption The well known fact that autoantibodies are present in both pernicious anemia and Sjögren's syndrome seems to suggest that the two diseases have a similar pathogenesis The relatively high rate of macrocytic anemia in RA (16) and of impaired vitamin B_{12} absorption in patients with RA (4) on the one hand and the frequent combination of RA and Sjögren's syndrome on the other corroborate this assumption No case report of megaloblastic anemia in association with Sjögren's syndrome is known to the authors

MATERIAL

Three patients with Sjögren's syndrome and vitamin B_{12} deficiency and one patient with Sjögren's syndrome with parietal cell antibodies and histamine refractory achylia were studied

Case No 1 Woman, 61 had been treated for RA since 1957 The disease had not caused severe destruction In 1967 polymyositis developed with marked symptoms from the skeletal muscles and high aldolase 18 6 i u (normal $<$ 5 i u) creatinephosphokinase 7 7 i u (normal $<$ 1 i u) and lactic dehydrogenase isoenzyme IV & V activities in serum A clearly positive biopsy specimen of muscle showed pronounced edema and perivascular infiltrations of lymphocytes and plasma cells The eyes and mouth were markedly dry Ophthalmologic consultation strongly supported the diagnosis of Sjögren's syndrome During hospitalization in 1969 the patient showed symptoms of psychic disturbance and later macrocytic anemia developed During vitamin B_{12} administration her psychic balance was completely restored and all blood values were normalized (Table I)

Case No 2 Woman 67 had been treated for severe progressive destructive RA since 1945 which had completely disabled her In 1958 she had a first episode of keratitis followed by several recurrences In 1968 she was admitted to hospital with severe macrocytic anemia (Table I) After B_{12} administration the peripheral blood picture returned to normal

Case No 3 Woman 54 (We were given access to this case by the Medical Department of the Central Hospital Kalmar [Head Karl Gydell M D]) developed symptoms of pernicious anemia in 1960 associated with a remarkable increase of the gammaglobulins in serum (Table I) Since then she has been on continuous vitamin B_{12} treatment which has completely restored the blood picture Since 1963 there have been many episodes of keratitis and dryness of the mouth No manifestations in the joints

Clinical Data of Three I[illegible] jogren's Syndrome and Vitamin B12 Deficiency and One Patient with [illegible]'s Syndrome and Signs of Gastric Lesion

		Case 1	Case 2	Case 3	Case 4
Schirmer's test		+	+	+	+
ESR	mm/H	117—15	50	68—100	109
Serum total proteins	g/100 ml	9	(1	8 8	9 2
Gammaglobulin	g/100 ml	3 14	1 5	4 28	3 6
Cryoglobulin		—	—	n t*	—
Waaler Rose titer		350	2800	256	1000
Latex fixation		+	+	+	+
LE cells		—	—	—	—
Anti Nucleic acid antibodies		—	±	n t	—
Anti intrinsic factor antibodies		Blocking + Binding —	Blocking —	Blocking + Binding —	Blocking —
Anti saliva protein antibodies		+	+	+	+
Immunofluorescence studies					
Anti nuclear antibodies		+	—		+
Anti parietal cell fluorescence		—	±	+	+
Anti thyroid antibodies				+	+
Anti myocardium antibodies		—		Cytoplasmic ±	
Bromsulphalein retention		12 5 %	n t	10 5 %	3 %
Before Treatment					
Hb	g/100 ml	7 0	4 8	The values are no longer available	12
E	mill /mm³	1 4	1 29	The values are no longer available	3 96
MCH	pg	35	34	The values are no longer available	30
Megaloblasts		— (the anemia developed rapidly during stay at hospital)	+	The values are no longer available	—
Schilling test		1 4 % II 0 8	1 1 2 % II 11 1 %	1 3 4 % II n t	23 6 %
Vit B12 in serum	pg ml	165	1900 (Vit B12 given before admission to hospital)		740
Folic acid	ng/ml	8	3 0	n t	3 4
Histamine refractory	achlorhydria	+	+	+	+
Serum Fe	µg/100 ml	21	112	116	4
TIBC	µg/100 ml	209	00	3 0	339
After B12 t[illegible] Treatment					
Hb					
E	g/100 ml	1 0			
MCH	mill /mm	3 8	11 4	1 5	
R[illegible]	pg	3	4 51	4 0	
[illegible]		[illegible]	24	35	
			[illegible]	1	
				135	

Case No 4 Woman 58 markedly elevated ESR and hypergamma globulinemia since 1967 Definite Sjogren s syndrome developed in 1968 In 1969 the blood picture was still normal in spite of positive antibodies to parietal cells and histamine refractory achylia

METHODS

The hemoglobin (Hb) red cell count (E) packed cell volume (PVC) erythrocyte sedimentation rate (ESR) and bone marrow were studied by routine methods The serum proteins were separated by paper electropho resis immuneoelectrophoresis and in two cases by ultracentrifugation The serum content of vitamin B_{12} was determined by microbiological assay with Euglena gracilis (1) and folic acid by the method of Gird wood (7) and 48 hour vitamin B_{12} absorption was tested by the method of Schilling et al (18) using a 1 μg dose of ^{58}Co labelled vitamin B_{12} and a single flushing dose of 1 mg unlabelled vitamin B_{12} two hours later (Schilling I) and with intrinsic factor (Schilling II)

Parietal cell antibodies were detected using the double layer immuno fluorescent technique and rat stomach (14) The method described by Samloff et al (17) was used to demonstrate intrinsic factor antibodies of blocking and binding type in serum and gastric juice The presence of antibodies to salivary protein in serum was tested by Ouchterlony s method (15)

The function of the lacrimal glands was tested by Schirmer s method The Bengal Rose test was used to detect corneal lesions

The Waaler Rose and latex fixation tests were used for the demonstra tion and titration of rheumatoid factor The double layer immunofluores cent method was used to detect antinuclear antithyroid and antimyo cardial antibodies

RESULTS

The concurrence of Sjogren s syndrome and macrocytic anemia due to vitamin B_{12} deficiency was demonstrated in three cases All these patients showed hypergammaglobulinemia and high titers of rheumatoid factor but no LE cells Three of the four patients described above showed antinuclear antibodies and antibodies to parietal cells In two out of four

sera investigated antibodies of blocking type to intrinsic factor were detected and in one out of two samples investigated similar antibodies were present in the gastric juice (Table I)

Precipitating antibodies to salivary protein were demonstrated in all four sera Antibodies to thyroid cell cytoplasm and myocardial structures were detected by immunofluorescence in a few instances (Table I)

DISCUSSSION

It has been conclusively shown that the rate of macrocytic anemia in rheumatics (4 16) is higher than expected Sjogren s syndrome is frequently associated with RA Therefore the concurrence of Sjogren s syndrome RA and vitamin B_{12} deficiency is not surprising Our material is too small to permit the conclusion to be drawn that the rate of this type of anemia in Sjögren s syndrome is higher than expected in this age and sex group

The case histories are suggestive of different courses probable RA — polymyositis — Sjogren s syndrome and vitamin B_{12} deficiency with anemia definite RA — Sjögren s syndrome — pernicious anemia vitamin B_{12} deficiency and anemia — Sjogren s syndrome These different combinations do not prove that Sjogren s syndrome and vitamin B_{12} malabsorption have the same etiology but they are evidence in favor of a similar pathogenesis in certain predisposed individuals Case no 4 is an example of Sjogren s syndrome with atrophic gastritis and antiparietal cell antibodies in serum The disease might terminate in megaloblastic anemia

Antibodies to parietal cells and to intrinsic factor have been found in sera from patients with pernicious anemia (20) thyrotoxicosis (13) insulindependent diabetes mellitus (21) and chronic gastritis In sera from patients with pernicious anemia parietal cell antibodies were observed in 83 % intrinsic factor antibodies of blocking type in 70 % and of binding type in 34 % (17) Our findings are in good agreement with these Only a few instances of intrinsic factor antibody in the absence of parietal cell antibody have been reported (12 22) Our case no 1 showed this combination

In gastric juice IgG antibodies of blocking as well as of binding type have been found (3) In addition an IgA with specificity for intrinsic factor has been detected (8) Antibodies to intrinsic factor were found

in the gastric juice of one patient in this series (Case no 1) Her Schilling I was 4 % and Schilling II 0 8 % The test with intrinsic factor was remarkably lower than without The presence of anti intrinsic factor antibodies in the gastric juice may play an important role preventing absorption of the B_{12} vitamin intrinsic factor complex

The relationship between antibodies to parietal cells and antibodies to intrinsic factor and disease is unknown The presence or absence of these antibodies does not correlate with the disease manifestations observed (10)

Feltkamp and van Rossum (6) detected antibodies to salivary duct cells in 16 out of 30 patients with Sjogren s syndrome They found antinuclear antibodies in 77 % and the Waaler Rose test was positive in 48 % No increased frequency was observed for parietal cell antibodies (6) By contrast three of the present four patients with Sjogren s syndrome showed antibodies to parietal cells The results hitherto published on the occurrence of these antibodies and their correlation to disease activity are not convincing The use of a leukocyte migration test in vitro to demonstrate cellular hypersensitivity directed against salivary glands is probably a more promising approach to this question (19)

The origin and effect of autoantibodies in pernicious anemia and Sjogren s syndrome remain unknown Antibodies to many different organs are often present in both pernicious anemia (11) and Sjogren s syndrome (6) The significance of these findings is uncertain but the clinical observations suggest a genetic and/or slow virus relationship with autoimmune consequences

The favorable clinical effect of glucocorticoids on the disease activity in Sjögren s syndrome is obvious Definite remission of pernicious anemia has been obtained by glucocorticoid administration (5 9) This may be interpreted as additional evidence in favor of a similar immunological mechanism in the two disorders

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TABLE I

Relationship of SLE to RA DLE and Sjogren s Syndrome

Basic disease	Combination	Time of onset of 2nd disease: Same time	2—5	6—10	11—15	16—20	Total	Total combined or alone	Total of other combination	Total cases
			Years later							
SLE	With RA	9	5	1	—	—	15			
	With DLE	10	1	2	—	—	13	31		
	With Sjogren	1	2	—	—	—	6		39	109
	Alone	—	—	—	—	—	—	36		
RA	With SLE	9	14	7	5	2	37			
	With DLE	1	2	2	1	1	7			
	With Sjogren	2	4	3	3	6	18	124		
	With LE cells	—	21	14	16	11	62		11	400
	Alone	—	—	—	—	—	—	265		
DLE	With SLE	10	5	1	1	—	20			
	With RA	1	1	2	1	—	5			
	With Sjogren	—	1	—	1	—	2	31		
	With LE cells	—	—	—	4	—	4		9	186
	Alone	—	—	—	—	—	—	146		
Sjogren s syndrome	With SLE	1	—	—	—	1	5			
	With RA		1	—	—	—	3	10		
	With LE cells	—	—	—		1	2		20	76
	Alone	—	—	—	—	—	—	46		
Total	Combined							199		
	Alone							493	79	771

CLINICAL INVESTIGATIONS

We established the diagnosis of SLE with the criteria selected by us and the diagnosis of DLE and Sjogren s syndrome on the basis of criteria accepted in the literature* For the diagnosis of RA we used the criterion of the American Rheumatism Association (2) In our survey only the cases satisfying the above criteria are included The same criteria were used in judging the different combinations of SLE RA DLE and Sjogren s syndrome

We have summed up the cases of 109 patients with SLE 400 with RA 186 with DLE and 76 with Sjogren s syndrome i e 771 patients altogether

We found different combinations of these four diseases in 278 patients out of 771 (Table I) Of the 109 patients with SLE 73 had combinations with the above mentioned three diseases SLE was alone only in 36 patients Among the 73 cases of SLE combined with other diseases there were 23 in whom RA DLE or Sjogren s syndrome appeared simultaneously with the onset of SLE In 39 cases SLE appeared 1—2 years after the onset of RA DLE or Sjogren s syndrome In 11 cases the classical RA Sjogren s syndrome or DLE cutaneous changes (in [illegible] cases RA in three DLE and in two Sjögren s syndrome) appeared 1—10 years after the clinical manifestation of SLE

The relationship of SLE and RA is even more conspicuous if we analyze our LE cell positive cases combined with classical RA So far we have seen 171 LE cell positive cases of these 109 were SLE and 62 LE cell positive RA In 43 out of 109 SLE patients classical RA was present In nine of these the classical RA appeared simultaneously with SLE in 28 some years before SLE and in six patients years after the onset of SLE That is to say that of the total number of 171 LE cell positive cases 105 i e 61 % had classical RA

Of the 109 SLE patients 23 had DLE In 10 cases the cutaneous changes appeared simultaneously with SLE in 10 some years before the onset of SLE and in three patients years after the onset of SLE

In our patients SLE RA DLE and Sjogren s syndrome were combined with each other in [illegible] % in this number RA and Sjogren s syndrome were combined with SLE or the other diseases in 39 % and DLE in 21 %

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* For criteria of diagnosis of SLE, DLE and Sjogren s syndrome see Appendix.

DISCUSSION

Investigating the course of SLE RA DLE and Sjögren's disease fo 15 years we found that there is close relationship between these fou diseases Sometimes symptoms of one or the other disease are prominent All combinations of the four diseases are possible In our patients they were combined with each other in 33 % Whether the actual diagnosis is SLE RA DLE or Sjogren's syndrome depends on the variability of the symptoms the monosymptomatic or polysymptomatic appearance the time of the investigation or the frequency of the examinations and the length of the observation period

On the basis of our investigations especially the relationship of SLE and RA is conspicuous This is exemplified by the fact that 61 % of all our LE cell positive cases were RA and in 40 % of the SLE patients classical RA was present Out of 400 cases of classical RA we found *LE cell positivity in 15 5 % Surveying all our LE cell positive cases we* found 36 % of them LE cell positive RA This is also very remarkable because the LE cell phenomenon — apart from a few cases — besides SLE occurs only in RA and because the positive LE cell phenomenon may be regarded as the absolute diagnostic criterion of SLE It is doubtful whether LE cell positive RA may be considered SLE and whether there is any difference between LE cell positive and LE cell negative RA

APPENDIX

Criteria Used in Diagnosis

SLE

A) *Results of Laboratory Investigations*

a) Positive LE cell phenomenon
b) Raised erythrocyte sedimentation rate
c) Dysproteinemia (lowered albumin increased gammaglobulin abnormal thymol turbidity)
d) False positive Wassermann reaction
e) Hematological changes (anemia leucopenia thrombocytopenia)

B) *Clinical Symptoms*

a) Fever (often septic) with negative hemoculture
b) Skin eruptions erythematosus rash on face
c) Raynaud's phenomenon
d) Hypersensitivity photosensitivity urticaria

e) Joint lesions (arthralgia polyarthritis of rheumatoid type)
f) Serositis or polyserositis (pleuritis pericarditis peritonitis)
g) Adenomegaly splenomegaly hepatomegaly
h) Visceral lesions (kidney lung heart, central nervous system gastro-intestinal tract pancreas liver)

C) An episodic clinical process and a favourable response to treatment with immune suppression (steroids Imuran)

For a definite diagnosis we required the positive LE cell phenomenon, the raised erythrocyte sedimentation rate and at least one other abnormal laboratory test and at least four clinical symptoms Our patients had been systematically reviewed every 3 to 6 months since 1954 to check the *episodic clinical process* and the effects of therapy

SJÖGREN S SYNDROME

A) Hypofunction of the lacrimal, nasal or salivary glands manifest by dryness of the eyes, mouth, nose larynx or skin sometimes scleroderma like with skin lesions and achylia
B) Parotid swelling
C) Arthralgia or polyarthritis of rheumatoid type
D) Raynaud s phenomenon
E) Photosensitivity

The degree of keratoconjunctivitis sicca (inflammation of the cornea and conjunctiva in consequence of dryness) was determined by Schirmer s method of measuring the tear secretion A filter paper 0 5 cm wide and 3—5 cm long was applied to the corner of the eye and the degree of humidity noted after 5 min The test was considered as positive only if the strip of paper moistened was smaller than 15 mm

For a definite diagnosis we required at least one of symptoms (B) to (E) besides the keratoconjunctivitis sicca (A)

DLE

A) *Chronic localized type*
focuses are on scalp and face (nose cheeks ears lips)

B) *Chronic disseminated type*
focuses are on neck, chest, back, hands

Chronic localized and disseminated types are
1 erythema
2 follicular hyperkeratosis
3 atrophy (in fresh cases possibly missing)

Histological characteristics are
1 follicular hyperkeratosis
2 atrophy of the Malpighian layer

3 degeneration of the basal cells as well as the collagenous and elastic fibers
4 infiltration of perivascular lymphocytes

In our cases the DLE diagnosis was established concerning the chronic localized and the chronic disseminated types

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INVOLVEMENT OF THE LATERAL ATLANTO AXIAL JOINTS AS FIRST AND LATE SYMPTOM OF RHEUMATOID ARTHRITIS

By

H van Kerckhove

Summary Thirty cases of unilateral or bilateral atlanto-axial arthritis are described They were found among 606 RA patients According to the degree of *involvement* the 30 cases were divided into four groups A 16 cases B 5 cases C 7 cases D 2 cases. In two patients the atlanto axial arthritis was the primary lesion The clinical and the radiological course of the disease is described The systematic search for this type of arthritis is emphasized as it is often asymptomatic The author gives details of a clinical test specific for the function of the atlanto-axial joints

We have studied 606 patients with RA among whom we found 30 people with clinical and radiological involvement of the lateral joints between the atlas and the axis This atlanto-axial (AA) arthritis ranged from a slight involvement to a destructive arthritis with luxation of the atlas or ankylosis of the upper part of the cervical spine

The purpose of this study is to show the existence of the AA rheumatoid arthritis to compare the evolution of this disease with RA itself to describe the clinical aspects of AA arthritis and to give a clinical test of this disease We will also describe two cases of RA where the AA arthritis was the primary location of this disease

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Selection of Patients

Between 1963 and 1967 we studied 606 patients with RA The diagnosis was established according to ARA criteria The patients who presented an *occipito-cervical pain or who had an impaired occipito-cervical mobility* at clinical examination received a special radiological examination We found 30 patients with these symptoms

Clinical Examinations

The occipito cervical area was examined as usual looking especially for the upper cervical mobility We investigated in particular frontal and lateral mobility the mobility of the spine when the head was in flexion or in rotation We also made a personal clinical test which proved to be specific for the involvement of the lateral AA joints

Specific Clinical Test of the AA Lateral Joints

We have defined a selective test for the AA lateral joints This test is based on the anatomo-physiologic function of those joints The efficacy and the selectivity of the test was proven in 300 of 606 patients

Technique The examinator stands in front of the patient and puts his left hand on the upper part of the patient s skull when his head is bent towards the right side for instance This hand blocks the right inclination of the head

The thumb of the right hand is placed under the right border of the chin and lifts the chin towards the left side to obtain a rotation of the head to the left With this test it is possible to check the mobility and the sensibility of the AA joints

By performing this test alternately with the head bent to the right or to the left side it is possible to check respectively the AA contralateral articulation *i e* on the left and on the right side Sometimes however it is the joint on the same side as the bent head that reacts The test may be explained on an anatomo physiologic basis If for example we consider that the head is bent towards the right the lateral inclination of the head *causes a rotation of the axis towards* the same side so that the superior articular facet of the axis slides backwards on the right side and forwards on the left If the head when in this position is turned to the left there is a rotation of the atlas on the axis (rotation between the occiput and the atlas being impossible for physiologic reasons) The rotation of the head towards the left displaces the inferior articular

TABLE I

Case no	Sex	Age	Stage	C1C2 test	X ray group	RAT	Serology WR	LE
7?79	F	43	II	2 +	C	2 ++	1/256	
? 98	F	59	III	3 +	A	neg	1/16	
?3?7	F	45	II	2 +	A	1 +	1/64	+
?390	M	53	III	2 +	C	3 +	1/64	
?987	M	29	II	1 +	B	neg	neg	
3?69	F	79	III	3 +	D	neg	neg	
4001	F	51	II	2 a 3 +	C	neg	neg	
4?83	F	45	III	3 +	C	1 +	neg	
4335	F	55	III	2 +	A	1 +	neg	
4363	F	56	III	1 +	A	3 +	1/?12	
454?	F	62	III	2 +	A	3 +	1/?048	
4(8?	F	67	III	3 +	C	3 +	1/?56	
5?74	F	68	I	1 +	A	1 +	neg	
5311	F	15	I	3 +	B	neg	neg	
5448	F	45	II	1 +	A	3 +	1/128	
5580	F	31	I	2 +	A	neg	neg	
5711	M	60	I	1 +	A	neg	neg	
5796	F	60	II	1 +	A	2 +	1/256	
5805	F	48	II	1 +	A	2 +	1/256	
5889	F	65	IV	3 +	D	3 +	1/512	
(00?	F	39	I	? +	A	neg	neg	
6?41	M	47	II	1 +	A	2 +	1/256	
6 74	F	53	II	2 +	B	2 +	1/256	
6555	F	55	III	1 +	B	neg	neg	
6654	M	69	III	3 +	B	3 +	1/512	
7344	M	53	IV	3 +	C	3 +	1/ 56	
7587	F	48	III	3 +	C	3 +	1/256	
76 9	F	55	I	3 +	A	neg	neg	
7887	F	55	I	1 +	A	neg	neg	
85?8	M	62	III	1 +	A	3 +	1/256	

surface of the atlas forwards on the right and backwards on the left side giving an opposite movement to what occurs in the axis

This opposite movement puts the articular surfaces between the atlas and the axis under strain and gives the possibility to appreciate the sensibility and the mobility of those joints

Radiological Examination

The patients who had a clinical AA arthritis or who had a positive test (see later) received a radiological examination of those joints The

principal examination was a transbuccal view Many doubtful cases also received this radiographic examination We can appreciate the clinical value of the test on our 300 patients examined For some patients the standard radiological x rays were not sufficient and we performed a radiokinetic study (40 patients) a tomographic study (21 patients) or a radiocinematographic study (16 patients) The radiocinematographic and radiokinetic examinations study the rotation the subluxation and the lateral displacement of the axis

Study of 30 Patients with AA Arthritis

Table I groups 30 patients with RA and involvement of the lateral AA joints We have the following characteristics

Sex Seven males and twenty three females This proportion shows a predominance of women but this is also the case for RA in general

Age The average was 52 years the youngest patient being 15 and the oldest 79 The average duration of RA was 8 66 years when the lesion was found Subdividing the patients according to Steinbrocker stages we find

Stage I	7	cases
II	9	,
III	12	
, IV	2	

Serology

Seventeen patients carried rheumatoid factor Thirteen were seronegative and in one patient we found rheumatoid factor and LE cells The proportion of 17 r f positive and 13 r f negative patients suggests that AA arthritis occurs more frequently in r f negative cases But it could simply be that AA involvement can be an early sign of RA We shall discuss this point later Another possible factor is that all the cases with proven RA received a gold salt treatment and this treatment retards the positive serology

Pain

Spontaneous pain was present in 21 patients In 11 cases it was on one side only In ten cases it was bilateral The pain is in the occipito cervical area We shall describe later the clinical aspects of this pain

The Clinical Test

The clinical test that we have already described forms a check of the pain and the mobility of the AA joints corresponding to the lateral flexion and the rotation that the head may obtain during the test To objectivize our results we use the following grading

Positive + The test gives a selective suboccipital pain (usually on the opposite side from the inclination of the head) and the mobility may be normal

Positive ++ The test causes a suboccipital pain and the mobility is impaired

Positive +++ The mobility is strictly impaired The inclination may be completely absent causing an ankylosing of the upper part of the cervical spine At this stage there may be no pain

Radiologic Groups

The 30 cases were divided into four groups according to the radiologic involvement of the atlanto axial joints

Group A Articular narrowing on one or two sides with impairment of the radiokinetic articular function

Group B Same characteristics as in group A but with erosion of the articular lining on one or both sides

Group C Complete obliteration of the involved joint and osteo destructive arthritis

Group D Same characteristics as in group C but with lateral luxation of the atlas

As shown in table I we have 16 cases in group A 5 cases in group B 7 cases in group C and 2 cases in group D

Radiologic Group A

Among the 16 cases in this group we found one case where the AA arthritis may be considered as the primary localization of RA It is a 31 year old woman (case 5580) consulting us for severe pain in the neck of six months duration Several treatments had given no help

The pain was spontaneous and the cervical mobility had always been normal The pain is located in the right suboccipital area The C1C2 test is positive on the right side SR 42 mm The hematocrite is normal and serum is negative for rheumatoid factor X ray shows a rotation of the axis with a slight pinching of the right AA joint (Fig 1) confirmed by tomography Radiocinematographic examination shows that



Fig 1 Roentgenogram showing a rotation of the axis with a slight pinching of the AA right joint

AA articular functions are absent while the lower cervical spine mobility is normal Three months later there appeared an arthritis of the right and left foot A short high dose of prednisone followed by gold therapy cleared the pain in the neck

The articular functions of the atlas and the axis were recovered clinically and radiologically Actually the disease is a moderate RA with peripheral localization Figs 2 and 3 show the rotation of the axis in the patient just described Radiocinematography proves that the rotation was absent and that there was a recovery of the mobility after three months treatment Pinching of the right lateral AA joint cannot be judged on this x ray because of the rotation of the axis but tomography confirms that there is slight pinching

Radiologic Group B

The five patients in this group show unilateral or bilateral pinching of the AA joints with loss of the articular function There is also erosion of the articular lining on the involved side

The principal case in this group is a young 15 year old patient who began to suffer from neck pain (case 5311) at the age of 12 years



Figs 2 and 3 The different rotation of the axis

The cervical spine mobility of this child had always been normal Some months later there appears gradually a classical juvenile polyarthritis Treatment did not cure the neck pain completely From the beginning the articular functions of the atlas and the axis were absent on cinematographic examination while the other cervical joints functioned normally



Fig 4 Case of group B On both sides there is a joint pinching with erosion of the articular lining on the right side

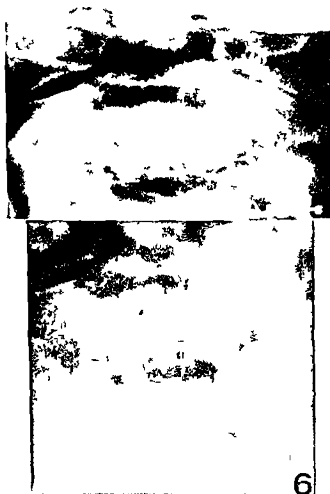
On both sides there was joint pinching with erosion of the articular lining on the right side (Fig 4) The first symptoms of this juvenile polyarthritis was the bilateral AA arthritis The further evolution of the disease was classical

Radiologic Group C

The seven patients in this group have unilateral or bilateral destructive osteo arthritis of the lateral AA joints

Two patients are typical for the group since on them we had the opportunity to perform a new radiological examination after an acute episode of arthritis in the AA joints In patient 2129 we see a severe osteo-articular destruction of the left AA joint when we compare the x rays 5 and 6 respectively dating from 1964 and 1967 During this period the patient presented several attacks of arthritis in the left AA joints

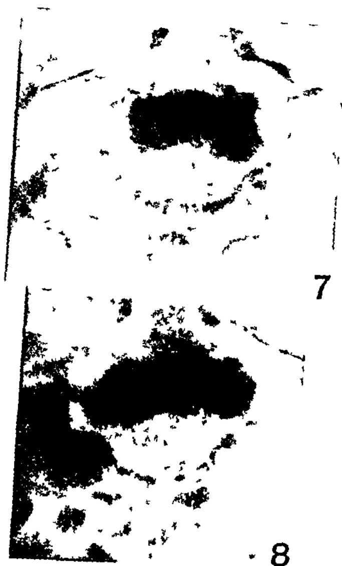
The second patient (cases 2590) had the same severe evolution of destructive arthritis on the right side between 1965 and 1966 (see figs 7 and 8) During this time the patient had a very severe attack of arthritis between C1 and C2 and he has now a slight rotation of the head



Figs 5 and 6 Roentgenograms taken in 1964 and 1967 showing a severe osteo-articular destruction of the left AA joint in 1967

Radiologic Group D

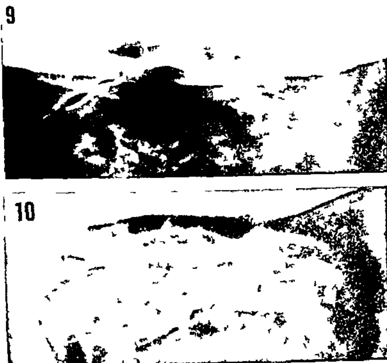
The two patients in this group have a destruction of the AA joints and of the ligaments causing a lateral luxation of the atlas Patient 3269 and patient 5889 both have a luxation of the atlas toward the right (figs 9 and 10) with almost complete destruction of the left AA joint



Figs 7 and 8 The sa e seve e ev lut n f de tru t e a l t s n the r ght side between 1965 and 1

Comp rison between le Rah l g d Inv ver ent f the 44 Joi s and the General J int In lvemen

Table II shows that the patients in radi l gic group D are in stages III and IV Patients in group C are in stages II III and IV wh le those in groups B and A are found in stages I II and III This sugges s



Figs 9 and 10 Roentgenograms of patients 3269 and 5889 both show a luxation of the left atlas towards the right with almost complete destruction of the left AA joint

that there is a correlation between the AA joint involvement and the involvement of the joints in general Usually the AA joints are attacked after the other joints except in two cases (5580 of group A and 5511 of group B) where the AA arthritis was the primary lesion

Clinical Evolution of the Atlanto Axial Arthritis

Most of the 30 patients complained of occipito cervical pain In the other patients the AA arthritis was detected through the clinical test already described In RA the AA arthritis can occur at any time as shown in table I but it is difficult to say when it really begins if one does not look for it systematically Clinically it is a unilateral or bilateral occipito-cervical pain covering about 10 square inches It is a dull continuous pain exacerbated by quick movements or fatigue of the cervical spine (car driving TV watching) There is no irradiation except in

TABLE II

Involv C_1C	7	9	12	2	Total
D			1	1	2
C		2	4	1	7
B	1	2	2		5
A	6	5	5		16
	I	II	III	IV	Stein brocker

severe cases where the pain extends towards the fronto parietal area Cutaneous sensibility of the occipito cervical area was always normal As the pain does not correspond to a specific nerve innervation area, it must be considered as a referred pain and not as a pain by nerve root irritation

There are however four exceptions to these general rules Two patients presented acute attacks of arthritis and two others had neurolog ical symptoms The first two are patients 2129 and 2390 in radiologic group C They presented at once an acute attack of arthritis in the AA joints calling for special treatment and leaving a definite inclination of the head in one patient X rays taken before and after the attack show an aggravation of the AA joint destruction (figs 5 6 7 and 8) Th neurological lesions were found in two patients of radiologic group D with lateral displacement of the atlas They presented periodically an acute attack of arthritis blocking the head completely as shown by radiological examination of the atlas During each attack there is a hyposensitivity with paresthesia of the posterior mandibular and occipito-cervical areas sometimes with a severe fronto parietal pain on the side most involved This is probably due to a mechanic or phlogistic irritation of the Occipitalis major nerve

The AA arthritis was treated according to the intensity of the pain and according to the radiological and functional involvement Patients of radiologic groups A and B were usually checked through the general treatment of RA and through focal physical medicine Groups C and D often required a cervical collar gentle controlled traction In very severe cases local peri articular infiltrations with procaine and steroids gave very good results

DISCUSSION AND CONCLUSIONS

While the atlanto odontoid arthritis is fairly well documented there are few studies on the involvement of the lateral atlanto axial joints We have shown that the involvement of these joints exist describing the clinical and the radiological pictures But there is one point unsolved when does AA arthritis begin during RA?

This is difficult to determine as many patients are only seen after several years of RA evolution If quite often there seems to be a correlation between the AA arthritis and RA itself as we have shown it is still possible that AA arthritis begins during the first period of RA but that treatment stops its evolution at least partially There are reasons for this hypothesis

1 Cases 5580 and 5311 present signs of AA arthritis several months before peripheral articular involvement

2 Considering RA as a whole only six of the 30 patients studied are in Steinbrocker s stage I while not less than 16 patients only have early signs of AA arthritis in group A (Table II) This proves that the evolution of the RA went faster than the AA arthritis but it does not mean that the AA arthritis began later

3 We found an important radiological and clinical involvement in several patients despite the fact that the patients did not require special treatment for this localization This proves that there must be a slow asymptomatic evolution

There is a last question why are 13 of the 30 patients sero-negative for rheumatoid factor since this is not the normal proportion of sero-negative and sero-positive cases in RA? Almost half of the cases are sero-negative This may be due to the fact that AA arthritis appears more frequently in sero negative RA or that the early gold therapy has retarded the appearance of rheumatoid factor

CONCLUSIONS

1 Among 606 RA patients 30 presented an atlanto-axial (AA) arthritis

2 A clinical screening test of the AA arthritis is described

3 In two patients the AA arthritis may be considered as the primary localization of RA

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4 According to the degree of involvement, the 30 cases of AA arthritis are divided into four groups according to the radiological involvement of the atlanto axial joints

5 The clinical course is described Two cases show, clinically an acute onset of AA arthritis and the x ray taken before and after this onset show the fast articular destruction

6 The treatment is the classical treatment of RA with physical medicine and peri articular infiltrations

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INHERITANCE OF RHEUMATOID ARTHRITIS

By

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Summary The inheritance of rheumatoid arthritis was investigated in three areas in Sweden where nearly the total population over the age of seven (22 707 persons) was examined for the prevalence of rheumatoid arthritis (ARA criteria) No significantly higher frequencies of rheumatoid arthritis were found among relatives of rheumatoid arthritics than among matched relatives of matched controls The results are disappointing and throw no new light on possible inheritance of rheumatoid arthritis

The purpose of this study was to find if there is a familial aggregation of RA in the total population In an area survey that was performed in 1962—1963 five different geographical areas in Sweden were investigated for the prevalence of RA In this study on inheritance three areas are included 22 707 persons being nearly a hundred per cent of the population over the age of seven (over 14 in Norrbotten)

MATERIAL AND METHODS

Total general populations over the age of seven (Norrbotten over the age 14) were examined as to the prevalence of RA in the following counties in Sweden a) Norrbotten 8 897 persons (3 916 males and 4 981 females) b) Kristianstad west coast 7 382 persons (3 613 males and 3 769 females) c) Kristianstad east coast 5 287 persons (2 571 males and 2 716 females) d) Re investigations of the primary non

response in Norrbotten and Kristianstad 1 141 persons (481 males and 660 females)

The distance between the northernmost and southernmost areas is about 1 500 kilometers

Rheumatoid arthritis was diagnosed in accordance with the criteria established by the American Rheumatism Association (16 19 29) Investigation routine practical aids in the investigation calculation methods statistically and by computer are described elsewhere as well as the reliability and validity of the results (1 16) Radiographs of the joints and serum determinations were if available collected from hospital records (16)

Total sampling technique was chosen for the primary investigations of the populations random sampling for children and systematic random sampling for the groups (10—20 per cent) not responding to the call for the primary investigation

Methods of Investigation of Inheritance

The family relationships for the persons investigated in Norrbotten and Kristianstad could be accurately determined by the aid of the parish registers of the Swedish Church (16) Traditionally a continuous registration of the population in Sweden has been made by the Church and in principle all persons belonging to the same household and family are registered in the parish record (forsamlingsboken) which contains each person s name date of birth address etc Thus the family relationships of persons investigated in the populations the names of all blood relatives etc could be established Blood relatives investigated were parents siblings children grand children

Relatives of persons with RA outside the areas chosen were not included as they were not investigated

Persons with RA (classical definite probable and possible) in the areas of Norrbotten and Kristianstad were matched with randomly selected control persons without RA of the same number sex age occupation and from the same geographical area as the patients with RA The relatives of the rheumatics and the controls included were matched as to comparable family relationships i e father of proband to father of control etc In the groups of different blood relatives of probands (i e fathers brothers etc) and in similar groups of blood relatives among the controls the frequency of RA was determined and the differences in the frequencies tested with the chi square test It was

TABLE I

Frequency of RA — Classical Definite and Probable — in Relatives of Persons with RA and among Relatives of Matched Control Persons in the Counties of Norrbotten and Kristianstad

Relatives	No	Relatives of Persons with RA		Relatives of Persons without RA	
		No RA	Per cent	No RA	Per cent
Fathers	27	0	0	1	3 7
Mothers	30	4	13 3	0	0
Sons	124	1	0 8	0	0
Daughters	96	1	1 0	0	0
Grand children, males	10	0	0	0	0
Grand children, females	12	0	0	0	0
Brothers	92	1	1 1	1	1 1
Sisters	75	5	6 7	3	4 0

watched that persons without RA matched to persons with this disease had no close family relationships between them

RESULTS

The results obtained are demonstrated in table I and II There is no significant familial aggregation of RA (classical definite probable and possible) in relatives of affected probands as compared to relatives of matched controls (Tables I and II) In table I possible arthritis is excluded The differences between the groups are not greater than would occur by chance

DISCUSSION

Previous studies may be classified broadly into family studies and twin studies No conclusive pedigree studies on RA have been carried

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CHANGES IN THE SYNOVIAL FLUID CAUSED BY OSMIC ACID

By

G LÖRINCZ H A ISOMÄKI and J MARTIO

Summary 27 rheumatic patients with knee joint hydrops were studied for the effect of osmic acid on the synovial fluid Special interest was taken in synovial leucocyte counts and electrophoretic patterns. A leucopenia preceded the leucocytosis in the synovial samples both were thought to be induced by osmium A precipitate formation from hyaluronate was thought to account for the leucopenia and this suggests a new hypothesis of osmic acid action In the electrophoretic patterns there was a change in the electric charges of proteins One week after osmic acid injection the pattern was normal

Reis and Swensson (10) have introduced intra acticular therapy with osmic acid as a method for chemical synovectomy Berglof (1) eliminated the frequent side effects of osmium tetroxide (OsO_4 osmic acid) in 1959 by adding hydrocortisone in this procedure Coagulation of the surface layer of the synovial membrane is caused by osmic acid but its effect can be followed also in the deeper layers of the synovial tissue (5 7)

In the available literature no mention was found about the early changes in the synovial fluid after osmic acid injection For that reason we have performed our study with special reference to alterations in synovial fluid leucocyte counts and electrophoretic patterns

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MATERIAL AND METHODS

The study involved 27 cases in all (24 rheumatoid arthritis, 1 Reiter's disease, 1 infectious arthritis and 1 ankylosing spondylitis), 16 women and 11 men, all with effusion in the knee. First the joint fluid was aspirated. This was followed by injection of 5 ml. of 1 % prilocain chloride and 10 ml. of 1 % solution of osmium tetroxide. Synovial fluid sample for investigation was taken from each knee joint before and at different time intervals after the injection. For in vitro investigation samples of 3 ml. joint fluid were aspirated and then immediately mixed with 1 ml. of 1 % OsO_4 in a glass tube. Prednisolone (10 mg.) was injected intra-articularly immediately after osmic acid, except in those cases in which the sampling was performed 5—10 minutes after osmic acid injection. In these cases prednisolone was administered after the sampling. In five in vitro samples the pH value was measured within five minutes by a Beckman apparatus and also by universal paper indicators. During the three months' trial 20 patients were injected with osmic acid once, six patients twice and one patient three times.

The synovial analysis was made of the fluid's total and differential leucocyte counts and electrophoresis. The samples were counted for total leucocytes in a hemocytometer. For differential counts smears were stained with Lofler reagent. After hyaluronidase treatment of the fluid the total proteins were determined by the biuret test (Weichselbaum's photometric method). The synovial fluid electrophoresis was accomplished by the Beckman Microzone System.

RESULTS

We have summarized our findings regarding the synovial fluid leucocyte counts in table I, in which, beside the mean values, the lowest and highest cell counts were also added. Although there was a marked deviation in single cell counts, the mean values demonstrated well the direction of changes. In the very early phase after osmic acid injection (5—10 min.) there occurred a striking drop of total leucocytes, both polymorphonuclears (PML) and mononuclear cells. Especially the number of PML decreased. Between one half and three hours the decreasing tendency of leucocytes continued when compared with the counts gained before osmium injection. On days 1—2 an obvious increase in all

TABLE I

Alterations in Leucocyte Count Caused by Osmic Acid

Time interval between injection and sampling	Total leucocytes		Polymorphonuclears		Mononuclears	
	Mean values	Deviation	Mean values	Deviation	Mean values	Deviation
Before injection	10 300	1 000—45 600	7 600	100—45 000	2 700	300—8 300
in vitro	2 400	100—7 700	1,300	50—5 200	1 100	50—3 300
5—10 minutes	1 300	0—4 400	700	0—2 700	600	0—1 700
½—3 hours	2 600	0—8 000	1 900	0—6 100	700	0—1 900
1—2 days	32 000	200—170 800	28 000	100—118 400	4 000	100—10 800
3—4 days	16 500	4 000—42 800	11 900	1 600—32 100	4 600	1 700—10 700
5—7 days	10 500	1 200—40 000	7 200	0—35 600	3 300	1 100—7 500

leucocytes followed mainly in PML numbers. On days 3—4 there was a slighter rise of these cells. During days 5—7 there was a fall in leucocytes again. The *in vitro* study also showed the decrease of leucocytes.

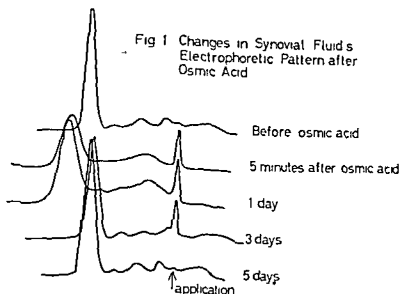
In the *in vitro* samples the pH values were 0.6—1.0 lower than the pH of the synovial fluid before osmium tetroxide treatment.

The results of the total protein determinations were recorded in table II. The lowest mean value was found 5—10 minutes after osmium te-

TABLE II

Changes in Total Protein Values of the Synovial Fluid Caused by Osmic Acid

Time interval between injection and sampling	Mean values (g%)
Before injection	4.7
in vitro	2.7
5—10 minutes	3.2
½—3 hours	4.1
1—2 days	5.5
3—4 days	4.7
5—7 days	4.2



troxide injection and the highest on days 1—2 The in vitro samples gave an average of 2 7 g%

On each synovial fluid specimen an electrophoretic study was performed In fig 1 some of the patterns gained before and at different times after osmic acid injection are shown The earlier the samples were taken after OsO_4 injection the greater were the changes in the pattern There were only two peaks to be found between five minutes and the third day after treatment The first peak was smaller than the usual albumin fraction ranged better and appeared somewhat sooner and most probably corresponded to albumin The second peak corresponded approximately to the beta position From the third day a little gamma fraction appeared again The pattern of albumin became higher and less wide and slowly returned to its appropriate field From the fifth day the recovery of the electrophoretic pattern was in progress Even on the fifth day a higher albumin and a smaller gamma fraction were visible but one week after the injection the pattern was normal

DISCUSSION

In the literature not much is mentioned of the mode of action of osmium tetroxide Most papers have described the mechanical coagula

ing effect on the surface of the synovial membrane Possibly the function of synoviocytes is also depressed (4) The slower osmium pool in the knee joint reported by Oka et al (7) can explain a therapeutic effect by the way of enzymatic inhibition Mottonen and his co workers (6) found a fibrin like exudation under the synovial intima in rabbits one hour after osmium injection Berglof (2) demonstrated patchy necrosis and rich leucocytic infiltration in the rabbit s synovium one day after osmic acid This could be a histologic explanation of the early toxic effect of osmium In human synovial tissue the corresponding acute in flammation decreased after one week.

We followed the changes in synovial leucocyte counts from the very early phase to the 7th day after osmic acid injection At first a diminution of leucocytes occurred which could be due to an effect of osmium It is a known fact, that before counting of synovial leucocytes the use of acidic diluent must be avoided otherwise a precipitate will be formed from the hyaluronate This precipitate traps the leucocytes and causes a marked decrease of them (3) The 1 % solution of osmic acid will most probably induce the same process which suggests a new theory of osmic acid s action Palade et al (cit ref 9) observed the decrease of pH during tissue fixation by osmium tetroxide In water-containing me dium cyclic osmic acid mono-esters are formed which possess acidic nature (9) Our therapeutic osmium tetroxide solution also reduced the pH of the synovial fluid The leucocytosis in the fluid came only after these beginning events here recorded from the first day

The changes in permeability and synovial fluid absorption were thought to account for its total protein level More recently the polymerisation state of hyaluronic acid was held to be responsible for this (3 8) In our study the mean total protein values showed almost the same trend of changes, as the cell counts

The alterations in the electrophoretic patterns are difficult to interpret It is evident that the electric charges are changed and possibly the posi tive charges are partly lost The otherwise neutral gamma globulins thus become electronegative begin to wander in the electric field and form a second peak on the curve How much denaturation of protein mole cules occur can not be derived from this study but decrease of amount of proteins in 5—10 min samples as well as in vitro is evident

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Acknowledgement

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MURAMIDASE (LYSOZYME) IN JOINT FLUID AND SERUM OF RHEUMATIC PATIENTS*

By

MARTTI OKA and OLAVI SEPPALA

Summary The muramidase activity of serum was studied in 27 cases of rheumatoid arthritis The enzyme activity in rheumatoid serum (mean 11 0 µg/ml) did not differ significantly from that in the 30 healthy controls (mean 9 4 µg/ml) The muramidase activity in the synovial fluid of 36 rheumatoid arthritis patients was markedly elevated (mean 20 2 µg/ml) In osteoarthritic synovial fluid (8 cases) the muramidase activity was low (mean 8 0 µg/ml)

No correlation was found between the total white blood count and the muramidase activity in rheumatoid synovial fluid A positive relationship was observed between the enzyme activity and the total protein concentration The elevated concentration of muramidase in synovial fluid of rheumatoid arthritis patients is interpreted as evidence of increased release of hydrolytic lysosomal enzymes It remains to be shown which type of synovial fluid leukocytes produces the enzyme in question.

Muramidase is a hydrolytic enzyme discovered by Alexander Fleming in 1922 in his own nasal mucus In Micrococcus lysodeikticus the most sensitive bacterial strain the substrate for muramidase is the cell wall mucopolysaccharide a complex material containing both sugars and peptides which is also called cell wall glycopeptide or peptidoglycan (25)

* Presented at the XIII Scandinavian Congress of Rheumatology Copenhagen June 9 1970

The simplest substrate on which muramidase acts is a tetrasaccharide NAG NAM NAG NAM where NAG=N acetylglucosamine NAM= N acetyl muramic acid Muramidase splits the middle link NAM NAG (26) Subsequently Salton proposed that lysozyme should be renamed muramidase and this has been accepted as the trivial name for beta 1-4 N acetyl glucosaminidases by the Commission of Enzymes

Muramidase can easily be isolated from hen egg white and purified and is a stable substance of rather low molecular weight (14 500) The complete primary structure of the enzyme was elucidated in 1963 (5 13) and the three dimensional structure in 1965 (2)

Most but not all mammalian tissues and secretions contain muramidase in varying amounts In the blood the enzyme has been demonstrated in mature neutrophilic granulocytes and monocytes (4) Serum muramidase activity has been found to be directly correlated with the absolute number of circulating mature granulocytes in normal subjects and in patients with neutrophilic leukocytosis and lymphocytic and granulocytic leukemia The highest muramidase activity has been demonstrated in monocytic leukemia (20)

Muramidase only appears in the urine when tubular damage occurs or when the serum muramidase concentration exceeds approximately three times the control level apparently demonstrating the existence of a renal threshold for excretion of the enzyme (10)

Rheumatoid inflammation and tissue damage have been supposed to result from the action of hydrolytic enzymes released into the synovial fluid from lysosomes of leukocytes and to a lesser extent from lysosomes of proliferating lining cells in the rheumatoid synovium (9) The hypothesis put forward by Hollander and coworkers (11) to account for the pathogenesis of rheumatoid joint inflammation presupposes the deposition of the rheumatoid factor — gamma globulin complex in the joint producing leukocytosis phagocytosis and release of lysosomal enzymes It has been suggested that a major pharmacologic effect of steroid hormones in diseases of connective tissue may be to protect the lysosomes against injury (31)

The large numbers of polymorphonuclear leukocytes are presumably the source of the various hydrolytic enzymes which are present at higher levels in rheumatoid synovial fluid than in normal synovial fluid or rheumatoid plasma These enzymes include acid phosphatase (27) alkaline phosphatase and transaminases (24) beta glucuronidase (17) pepsin and trypsin (29) An increased activity of acid phosphatase (1

17 30), cathepsin (17) and leucinoaminopeptidase (28) has been demonstrated in the synovial membrane At the same time the number of lysosomes increases greatly (1) Enzymes capable of splitting chondromucoprotein have also been found in human articular cartilage (6 16)

Muramidase has been identified in synovial lining cells (23) A relationship between antinuclear factor and muramidase activity has been shown in rheumatoid sera but not in sera from patients with SLE (21) It has been suggested that a positive test for ANF in patients with RA may reflect inadequate binding of endogenous muramidase in serum (72)

The aim of the present investigation has been to study the activity of muramidase in synovial fluid and serum of patients suffering from RA Muramidase in the synovial fluid of patients with osteoarthritis has also been examined.

MATERIAL AND METHODS

Muramidase was estimated from 44 joint fluid samples 36 of which were obtained from RA patients and 8 from patients with osteoarthritis Serum muramidase was determined in 27 sera drawn from RA patients 30 healthy persons from the laboratory staff served as controls

The studies were performed from fresh samples of joint fluid and serum To prevent coagulation potassium oxalate was placed on the bottom of the test tubes The muramidase concentration was measured by the turbidimetric method of Litwack (14) A fresh suspension of heat killed Micrococcus lysodeikticus organisms in buffer was rapidly mixed with a test sample in a cuvette and placed in the pathway of the light Muramidase activity was measured by the rate of change of optical density and its activity was expressed as micrograms of crystalline egg white muramidase per milliliter of sample Any sample with a muramidase activity of 20 μg/ml or more was retested after dilution This was necessary because of the poor relationship between changes in optical density and muramidase concentrations at higher levels of enzyme activity

The relationship between muramidase activity and circulating or synovial fluid leukocytes was expressed as an index This value — the muramidase index — was determined as follows

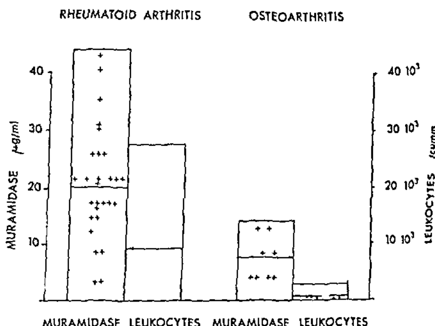


Fig 1 Muramidase activity (+) and total leukocyte count ( ) in synovial fluid of patients suffering from rheumatoid arthritis or osteoarthritis

$$\frac{\text{muramidase } \mu g/ml}{\text{leukocytes per cu mm}} \times 1\,000$$

An elevated index has been interpreted as indicating an increased turnover or increased enzyme content of leukocytes

RESULTS

The results are presented in figs 1 and 2 In rheumatoid *synovial fluid* the muramidase activity varied from 1 to 43 µg/ml (average 20 2) The number of leukocytes in the synovial fluid of these cases varied from 1 000 to 26 500 per cu mm (average 9 800) In osteoarthritic synovial fluid the muramidase activity was low varying from 5 to 13 µg/ml (average 8 0) The synovial fluid leukocytes in the corresponding cases varied from 170 to 2 100 per cu mm (average 800)

The muramidase index was elevated in rheumatoid synovial fluid

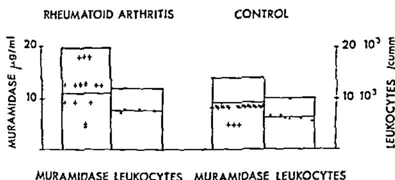


Fig Muramidase activity of serum (+) and total white blood count ( ) of patients suffering from rheumatoid arthritis and healthy controls

(average 2 2) and strongly elevated in osteoarthritic synovial fluid (10 0) The index was about the same in the rheumatoid serum (1 4) and the control serum (1 5) No correlation was found between the total number of leukocytes and the muramidase activity in rheumatoid synovial fluid

The muramidase activity in the cases in which the synovial fluid gave a positive latex test averaged 18 8 µg/ml the corresponding value in the latex negative cases being 23 3 the difference is not statistically significant On the other hand the synovial fluid muramidase activity was found to be related to the total protein concentration of the joint fluid in RA The enzyme activity was significantly higher (average 25 5 µg/ml) in the cases with a protein concentration of 5 g/100 ml or more than in those with a protein concentration of less than 5 g/100 ml (average 16 3 µg/ml) ($t = 3 0$ $p = 0 01$)

The difference was highly significant when osteoarthritis cases were included ($t = 3 8$ $p = 0 001$)

The muramidase activity in the *serum* of RA patients ranged from 4 to 18 µg/ml (average 11 0) The total white blood count ranged from 4 300 to 11 400 per cu mm (average 7 600) The mean value of muramidase activity in rheumatoid synovial fluid was twice as high as in the serum In the control serum the muramidase activity ranged from 5 to 13 µg/ml (average 9 4) The total white blood count ranged from 4 100 to 9 900 per cu mm (average 6 050) The muramidase activity in rheumatoid serum did not differ significantly from that of control seru

DISCUSSION

The results presented show clearly that the synovial fluid of patients with RA contains increased amounts of the enzyme muramidase. A certain amount of the synovial fluid muramidase obviously originates from the circulating blood being dependent on the actual enzyme activity in the serum and the changes in synovial membrane permeability. However, it can be suggested that most of the excess enzyme activity in the joint fluid originates from the synovial fluid leukocytes. According to the present knowledge the release of hydrolytic lysosomal enzymes in the joint is an integral part of the rheumatoid disease process. The finding of increased muramidase concentrations in rheumatoid synovial fluid is a reflection of this phenomenon.

Whether muramidase plays an important role in the breakdown of connective tissue polysaccharides in RA is questionable. Muramidase is capable of splitting terminal glucosaminidic residues (3).

The elevated muramidase index in rheumatoid synovial fluid can be interpreted as evidence of an increased turnover of synovial fluid leukocytes. On the other hand, it is not excluded that the enzyme content of synovial fluid leukocytes could also be increased as a sequel to an increase in the number of lysosomes. What proportion of the synovial fluid muramidase originates from monocytes was not subjected to study. No correlation was found between the total number of leukocytes and the muramidase activity in rheumatoid synovial fluid.

A relationship was found in our study between the total protein concentration and the muramidase activity in synovial fluid. Mäkinen (18) observed that the total protein concentration and the colloid osmotic pressure tended to be higher in rheumatoid fluids with large effusions. It has been suggested that there must be a disturbance in the disappearance of proteins from the joint cavity in these cases (19). It is possible that the disappearance of muramidase from the joint cavity is likewise dependent on the amount of synovial fluid. Latex positivity was not found to have a significant effect on enzyme activity.

The synovial fluid of patients with osteoarthritis contained markedly lower activities of muramidase than rheumatoid joint fluid. The enzyme activity was lower than that in the control serum. It is quite possible that most of the muramidase in osteoarthritic joint fluid is an ultrafiltra-of the serum enzyme. As we know, muramidase is a protein of low

molecular weight It is largely unbound in plasma and readily filtered by the glomeruli (8 15)

Acknowledgement

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EFFECT OF NON STEROIDAL ANTI INFLAMMATORY DRUGS ON OSTEOARTHRITIS OF THE KNEE

With Special Reference to PSP Clearance as an Indicator

By

H Asai and R Nakamura

Summary PSP clearance of the osteoarthritic knee was studied in 79 joints of 60 patients Correlation between the clearance rate and the degree of disease activity (the grade of inflammatory exacerbation) was suggestive

Using PSP clearance we studied the effect of non steroidal anti in flammatory drugs on the osteoarthritic knee The results of this study are as follows The drugs phenylbutazone, indomethacin and acetyl salicylic acid, are effective when they are administered at the initial phase of inflammation while in the chronic phase the effects of them are variable though significant in patients whose PSP clearance rate is below 45 per cent In the chronic and severely affected cases the basic principles of therapy of osteoarthritic knees are avoidance of excessive use and the correct use of rest and physical therapy

Osteoarthritis is not primarily regarded as an inflammatory process but the inflammation often enters into it as a secondary feature One of the important problems in the osteoarthritic knee is an exacerbation of the inflammation with synovial effusion Intra articular injection of corticosteroids and oral administration of anti inflammatory drugs are often effective in this phase The studies were already widely done about the intra articular corticosteroids in the osteoarthritic knee (4 5 11 14)

This investigation was designed to determine the effect of non steroi

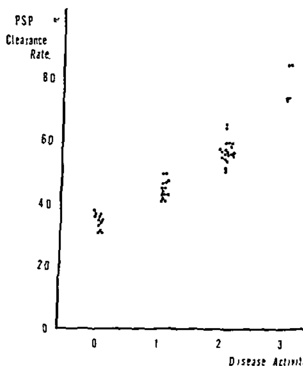


Fig 1 Correlation between PSP clearance rate and disease activity

al anti inflammatory drugs on the osteoarthritic knee Moreover an endeavor was made to correlate PSP (Phenolsulphthalein) clearance changes with the relief of symptoms in the affected joint and to establish an objective method for drug trials

Part 1 PSP Clearance Rate in Osteoarthritis of the Knee

MATERIALS AND METHODS

Sixty patients whose complaints arose from exacerbation of inflammation in the osteoarthritic knee were included in the study Both knees were used in 19 cases and a total of 79 joints were investigated No patient had received any treatment before the test within two weeks

PSP clearance rate This was assessed in terms of the urinary dye excretion rate at three hours after the intra articular injection of 1 ml PSP This method was previously reported in detail (8)

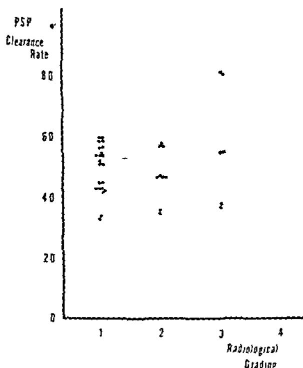


Fig 2 Correlation between PSP clearance rate and radiological changes

Disease activity This was graded from 0 to 3
0 = None
1 = Minor (*Heat — Tenderness ± Pain at rest — Pain on use ±*)
2 = Moderate (Heat — Tenderness + Pain at rest — or ± Pain on use +)
3 = Severe (Heat + Tenderness + Pain at rest ± or + Pain on use +)

Radiological changes The radiological appearances were graded into four categories for osteoarthritis (12)
1 = *Doubtful (Doubtful narrowing of joint space and possible osteophytic lipping)*
2 = Minimal (Definite osteophytes and possible narrowing of joint space)
3 = Moderate (Moderate multiple osteophytes definite narrowing of joint space and some sclerosis and possible deformity of bone ends)

TABLE I

PSP Clearance Rate for each Group of Disease Activity

Disease activity	No of cases	Mean	Standard deviation	Range
0	16	32 6	4 3	23 0—38 0
1	27	45 5	4 0	39 0—51 0
2	27	58 3	4 1	51 0—66 6
3	9	78 7	4 9	71 6—85 0

4 = Severe (Large osteophytes marked narrowing of joint space severe sclerosis and definite deformity of bone ends)

RESULTS

The results are presented in figs 1 and 2 where the amounts of PSP excreted in three hours after the intra articular injection are ex pressed in per cent The analysis of these figures may be summarized as follows The correlation between PSP clearance rate and disease activity was suggestive while that between PSP clearance rate and radiological changes was non significant PSP clearance rate for each group of disease activity is presented in table I

DISCUSSION

These findings confirm our previous observation that PSP clearance from the knee may be used to measure the degree of disease activity in osteoarthritis PSP clearance rate closely relates to the grade of in flammation (8)

The clearance studies in knees were done by several workers usi rheumatoid arthritis and osteoarthritis Harris and others (3) reported that radiosodium clearance rate for knees in rheumatoid arthritis shown more severe local disease activity was generally higher Also Dawson and Wisham (1) reported the increased radiosodium clearance rate in the osteoarthritic knee and the decreased clearance with relief of pain

Since the clearance rate depends on the local blood flow and the cap illary permeability the increased clearance rate is the result of increase

local blood flow and permeability According to Harris (2) the hyperemia of inflammation, which leads to a high rate of sodium clearance decreases as the local disease settles and the sodium clearance returns towards normal values Also one of the most characteristic aspects of inflammation is the increased capillary permeability Thus the increased PSP clearance rate should indicate the intensity of local inflammation

Part 2 PSP Clearance in Osteoarthritis of the Knee as an Assay for Anti Inflammatory Drugs

The synovial fluid analysis is seldom helpful for grading inflammation in the osteoarthritic knee The joint inflammation is only assessed by means of such parameters as walking time tenderness pain and number of analgesic tablets (13) These methods are easy and accurate but are rather insensitive in short term drug trials (7)

As shown in Part 1 PSP clearance measurement is a simple objective and reliable method for grading inflammation in the osteoarthritic knee We applied this method to evaluate the effect of non steroidal anti inflammatory drugs for the osteoarthritic knee

MATERIAL AND METHODS

Thirty patients with inflammatory exacerbation of the osteoarthritic knee were included in the trial All were ambulant and complained of pain tenderness and swelling of varying degree in the affected joint No patient had received any therapy within two weeks prior to the start of the trial

Degree of disease activity and PSP clearance rate were measured before and 5 to 10 days after the course of treatment with drugs The following drugs and dosages were used

Phenylbutazone 200 to 300 mg two or three times daily
Indomethacin 50 to 75 mg two or three times daily
Acetylsalicylic acid 2 to 4 gms three or four times daily

In this trial we arbitrarily divided the subjects into two groups according to PSP clearance rate Group A whose PSP clearance rate was below 54 per cent and group B above 55 per cent since the upper limit of PSP clearance rate in group 1 disease activity (Minor) was 54 per cent

The follow up study was continued for 10 weeks in three chronic and severely affected cases

TABLE II

Effect of Drugs on Disease Activity and PSP Clearance in Acute Phase

Cases	Before		Drugs		After	
	Disease activity	PSP clearance %	Doses	Duration	Disease activity	PSP clearance %
M 62	2	66	PB 300 mg	5 days	1	46
M 45	2	52	PB 300 mg	10	0	40
M 60	2	62	IM 50 mg	10	0	37
F 55	1	50	IM 50 mg	7	0	41
F 60	1	47	ASA 2 5 g	5	0	38
F 63	1	47	ASA 3 0 g	7	0	37

(Abbreviation used Phenylbutazone = PB Indomethacin = IM Acetylsalicylic acid = ASA)

RESULTS

The results of this trial are presented in table II Clinical improvement and decrease in PSP clearance rate were observed in patients who received drugs within two days (acute phase) after the onset of inflammation though the complete remission was not obtained in severely affected cases (Table II)

In this trial we did not attempt to find the dose response of drugs and could not find any difference of effect among three drugs

The response to drugs in chronic cases which received drugs more than a week after the onset of symptoms was variable However the effect of treatment in patients whose PSP clearance rate was below 54 per cent (Group A) was significant (Fig 3) Most of severely affected

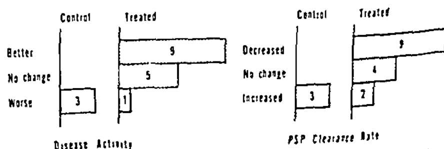


Fig 3 Effect of drugs on disease activity and PSP clearance rate in chronic phase of osteoarthritic knee of group A (minor inflammation)

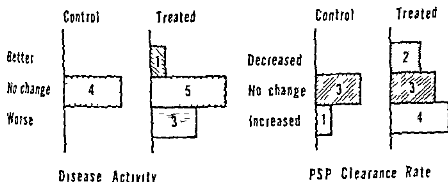


Fig 4 Effect of drugs on disease activity and PSP clearance rate in chronic phase of osteoarthritic knee of group B (moderate or severe inflammation)

patients whose PSP clearance was above 55 per cent did not improve within 10 days (Fig 4)

Being prescribed the avoidance of excessive use and the non weight bearing quadriceps exercise three patients improved 4 weeks after the start of treatment Complete remission was observed 10 weeks later (Fig 5)

DISCUSSION

The result of this study shows that the inflammatory aspects are often observed in osteoarthritis of the knee Although medications are usually given for the control of pain and stiffness in osteoarthritis at the present time, drugs should also be used to alleviate the inflammatory process

Non steroidal anti inflammatory drugs are usually effective in the osteoarthritic knee when they are used at the beginning of inflammatory exacerbations Both the disease activity and PSP clearance rate decrease with these drugs Sharp (2) reported that investigating the permeability of synovial membrane in the rabbit the decrease in membrane permeability was a common pharmacological property of the anti arthritic compounds Harris and others (3) reported that improvement of the local disease activity was accompanied by a decrease in the rate of clearance of radiosodium whether the improvement was spontaneous or brought about by intra articular injection of hydrocortisone

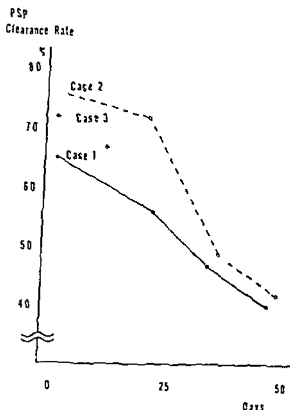


Fig 5 Follow up study in three chronic and severely affected patients They were prescribed daily rest and non weight bearing quadriceps exercise Following drugs were used Case 1 male 69 year old phenylbutazone 300 mg Case 2 female 65 year old indomethacin 75 mg and Case 3 female 38 year old acetylsalicylic acid 3 5 g

However the effect of these drugs both on disease activity and PSP clearance rate are not constant when they are administered at the chronic phase of inflammation Using acute experimental synovitis in dogs Rosenthale and others (9) reported that when doses that were completely effective by the pretreatment schedule were given at maximum symptoms complete suppression of the inflammatory response was not obtained and greater sensitivity was obtained when a compound was given before rather than after the experimental inflammation

From these facts we conclude that the relief of symptoms in the osteoarthritic knee is easily obtained with non steroidal anti inflam-

matory drugs when the medications are started at the initial phase of inflammation

In the chronic phase the basic principles of treatment of the osteoarthritic knee as Jeffrey (6) states are avoidance of predisposing or aggravating factors and correct use of rest and physical treatment By this method even severely affected patients could be improved within 10 weeks from inflammatory exacerbation whether the therapy consists of non steroidal anti inflammatory drugs or intra articular corticosteroids

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RELATIONSHIP BETWEEN IgG COMPLEXES AND ANTI IgG ANTIBODIES IN RHEUMATOID ARTHRITIS

By

EINAR MUNTHE

Summary A selective concentration of antibodies to IgG, both IgM and IgG rheumatoid factors and antibodies to the pepsin site of IgG, was demonstrated in eluates of rheumatoid tissues, but not in control tissues. Many eluates with high amounts of IgG complexes lacked free antibodies to IgG, even when these antibodies were present in the serum. This indicated blocking by IgG complexes Thus IgM rheumatoid factor could evade detection in some seronegative patients by fixation to tissue IgG complexes

INTRODUCTION

Tissue eluates from nephritic kidneys of systemic lupus erythematosus have been valuable in the study of tissue bound antibodies and immune complexes (6) In rheumatoid arthritis (RA) complexes of IgG are detected in sera (7) and in joint fluids (29) Eluates from rheumatoid synovial membranes and nodules also contain high molecular weight complexes of IgG (14) After pepsin digestion these complexes revealed IgG rheumatoid factors (15 16) and immunoconglutinins (9) Furthermore, free antibody activities against IgG were encountered in many rheumatoid eluates particularly if such antibodies were present in the co

responding sera in high titers (15) The present investigation was undertaken to study the relationship between eluate IgG complexes and anti IgG antibodies in eluates and sera from patients with rheumatoid arthritis and variants

MATERIALS AND METHODS

Human sera

Sera from RA and control patients were collected not more than three weeks before and at various intervals after synovectomy stored at —20°C and heat inactivated (56°C for 30 min) to destroy heat labile complement components before being tested

Rabbit antisera

Antisera against plasma proteins and complement components were produced absorbed and tested as previously described (15)

Tissues

Synovial membranes obtained from consecutive operations to the extent these provided sufficient material were from 78 patients with rheumatoid arthritis and variants classified according to the A R A criteria.

The membranes were taken from various joints (Table I) and in 15 cases from different joints in the same patient Operations were mostly performed in bloodless fields The interval beetween first and last operation on each patient was not more than nine months Subcutaneous nodules and a rheumatoid meningeal granulation tissue which caused erosions in the cervical spine were also tested Control tissues were from patients with non rheumatoid synovitis or degenerative joint diseases Histological classification of the tissues was kindly performed by the Biopsy Department Rikshospitalet

Eluates

Just after removal pure rheumatoid tissue was rinsed cut into small pieces and either used immediately or stored at —70 C In some cases it was lyophilized and stored at —20 C Elution procedures were applied on homogenized (Sorvall omnimixer) or disintegrated (X press Bio-

— 5

TABLE I

*Joints Providing Rheumatoid Synovial Membranes *)*

Groups	No of tissues from							
	Knee	Baker cyst (knee)	Wrist	Elbow	Hip	Ankle	MCP	MTP
Seropositive RA	32	5	20	6	1	1	1	
Seronegative arthritis								
a Definite rheumatoid	10	0	4	0	0	0	0	0
b *Atypical rheumatoid*	2	0	1	0	0	0	0	0
c Juvenile rheumatoid	1	0	1	0	1	1	0	0
d Psoriatic arthropathy	5	0	1	0	0	0	0	0
e Ankylosing spondylitis	1	0	0	0	0	0	1	0
Total	51	5	27	6	[illegible]	2	[illegible]	[illegible]

* The joints were in anatomical stages II—IV according to the A R A criteria RA Rheumatoid arthritis MCP Metacarpophalangeal joint MTP Metatarsophalangeal joint

Tec AB or Branson sonifier) tissue which was washed ten times in ten times excess of phosphate buffered saline pH 7 2 (PBS) and spun down at 15 000 x g The temperature in the tissue was kept below 20°C to avoid denaturation during these procedures The last supernatant was always clear and did not contain detectable immunoglobulins or antibody activities after twentyfold concentration Eluant was added volume per volume of wet tissue and elution was performed by stirring at room temperature for 60 minutes Citrate buffer of 0 1M and pH 3 3 2M NaCl and 3M NaSCN were each tried as eluants Elution was also carried out with PBS at 56°C for 30 min The different elution procedures gave total protein concentrations and anti gamma globulin titers in the same ranges However as elution with 3M NaSCN after tissue disintegration in the X press regularly gave the highest yield of IgG this treatment was adopted as the standard method Eluates were dialysed against PBS treated with hyaluronidase usually heat inactivated and filtered to remove floating fat (15) By this method reproducible results were obtained with different eluates from the same tissue For control purified native IgG was run through the elution procedure However no change in its inhibition pattern in the latex fixation inhibition test was found

Immunodiffusion tests

Immunoelectrophoresis was performed in 1 % agar with barbital buffer of ionic strength 0 025 pH 8 6 and 0 05 % sodium azide Double immunodiffusion was performed in 0 5 % agarose with PBS containing 0 5 % dextran and 0 1 % sodium azide Holes were cut 4 mm in diameter and 8 mm from centre to centre IgM rheumatoid factor and heat aggregates of IgG were isolated as earlier described (15) Radial immunodiffusion for quantitation of IgG and IgM was performed with monospecific antiserum in the gel and IgG and IgM in a normal serum pool as standards (see 15)

Protein concentrations

Total protein concentrations were measured by a modified Folin method (see 15) and in some cases also by the micro Kjeldahl method Serum gamma globulin concentrations were roughly calculated after total protein determinations and electrophoresis in cellulose acetate The electrophoretic protein patterns were scanned for light absorption with interference linefilter 546 nm in a Vitatron photometer

Agglutination tests

Rheumatoid factors were detected by red cells sensitized with anti Rh Ripley by Hyland RA latex slide test (26) and by a modified Waaler Rose test using rabbit antibodies to human red cells (20) The sensitivity of the test systems was adjusted to give less than 5 % positive reactions in a panel of normal sera To compare the sensitivity of the anti Rh Ripley and the Waaler Rose test some eluates and the respective sera were simultaneously titrated in both systems The titers were never more than two titration steps higher in the former test

Rheumatoid factors with anti Gm specificity were detected by red cells coated with specific Gm (a) (b) (f) and (g) anti Rh antibodies respectively (18)

Antibodies to the pepsin site of IgG (pepsin agglutinators) were detected by red cells sensitized by pepsin digested anti Rh antibodies (17) Tests for anti A and anti B isoantibodies and antibodies to rabbit red cells were performed by slide technique (27)

Patients with serum rheumatoid factor titer below 16 in the anti Rh Ripley modified Waaler Rose and latex fixation tests on repeated examinations before synovectomy were classified as seronegative

Inhibition test for detection of IgG complexes

The latex fixation inhibition test for detection of IgG complexes was performed with one drop of IgM rheumatoid factor serum 429 diluted 1 1 000, one drop of test sample and one drop of latex reagent diluted 1 16 in glycine buffer as previously described (15)

Test for anti nuclear antibodies

Anti nuclear antibodies were detected by incubating one drop of serum or eluate with acetone fixed mouse liver cryostat sections for 30 min at room temperature in a moist chamber After washing for 3 x 10 min, the sections were incubated with fluorescein labelled anti human gamma globulin for 30 min washed and inspected by microscopy in ultraviolet light

Reduction, alkylation and pepsin digestion

Reduction was carried out in 0 1M 2 mercaptoethanol (Type I Sigma Chemical Co) and alkylation in 0 02M iodoacetamide (Fluka AG) (28) Pepsin digestion was performed at an enzyme to-substrate ratio of 2 100 (w/w) in 0 1M acetate buffer pH 4 1 (17) The splitting of IgG was demonstrated both in immunodiffusion by loss of Fc antigens (Fig 4) and by testing for Gm antigens as previously described (15)

RESULTS

Antibodies to IgG

Free rheumatoid factor activity was present in 59 % of synovial membrane eluates from seropositive patients (Table II) while such activity was present only in 10 % of eluates from seronegative patients Antibodies to the pepsin site of IgG (pepsin agglutinators) were detected in about 50 % and 14 % of synovial eluates from seropositive and seronegative patients respectively These antibody activities were not due to serum contamination since isoantibodies and heterophile antibodies of IgM and IgG type were not detected in the eluates despite their presence in high titers in the corresponding sera Both the rheumatoid (Fig 1) and control joint tissue eluates contained IgG Control eluates did not contain antibodies to IgG but some had total protein and IgG concentrations which were as high as those of rheumatoid eluates

TABLE II

Protein Concentrations free Anti IgG Antibodies and Inhibitor for IgM RF) in Tissue Eluates*

Groups	No of patients	No of eluates**)	Mean prot conc (mg/ml)	No of eluates with free RF	PA	No of eluates with inhibitor for IgM RF
Synovial membranes from						
1 Seropositive RA	53	68	5 6	40	21	51
Seronegative arthritis						
a Definite rheumatoid	1?	14	3 4	0	4	9
b Atypical rheumatoid	3	3	7 3	0	0	3
c Juvenile rheumatoid	4	4	4 4	1	0	1
d Psoriatic arthropathy	4	6	6 5	2	0	5
e Ankylosing spondylitis	?	2	2 0	0	0	1
3 Controls	1	12	1	0	0	0
Rheumatoid nodules from						
Seropositive RA	5	6	1 4	5	0	
Seronegative RA	1	2	1 0	0	0	
Meningeal tissue from						
Seropositive RA	1	1	2 0	0	0	1

RA Rheumatoid arthritis RF Rheumatoid factor PA antibodies to the pepsin site of IgG (pep in agglutinators)

*) Measured by the latex fixation inhibition test

**) Each tissue is represented by the eluate giving the highest protein yield

Eluates from subcutaneous nodules of seropositive patients were frequently positive for rheumatoid factor while eluates from nodules of a seronegative patient and from meningeal tissue were negative None of these eluates contained free antibodies to the pepsin site of IgG

The titers of antibodies to IgG were usually low compared to those of the corresponding sera (Table III fig 5) Antibodies to the pepsin site of IgG were present in sera from 87 % of the seropositive and 38 % of the seronegative patients These antibodies were not detected in eluates when they were lacking in corresponding sera Prozones were observed in some eluates both by titration of rheumatoid factors and antibodies to the pepsin site of IgG The rheumatoid factor activity disappeared from all except three eluates after reduction and alkylation

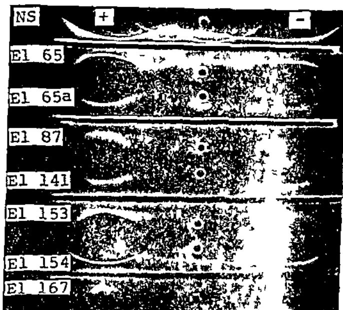


Fig 1 Immunoelectrophoresis of 7 rheumatoid eluates and normal human serum (NS) against rabbit anti human serum Eluates show mainly two precipitation lines one for albumin and one for IgG All eluates except 87 showed indications of IgG complexes but only 87 and 154 had free rheumatoid factor

When eluates were tested for rheumatoid factor in Waaler Rose and anti Rh Ripley and latex fixation test the results showed good correlation However the Waaler Rose test was negative in a few cases where the anti Rh Ripley and the latex fixation tests were positive Several eluates with rheumatoid factor titers of 64 or higher precipitated heat aggregated IgG on agarose plates

Rheumatoid factors with anti Gm activity were found in eight out of 32 rheumatoid factor positive eluates tested for this activity while 13 of the patients had such activity in the respective sera In two cases the anti Gm activity detected in the eluates was not found in the corresponding sera

Antinuclear antibodies

Weak anti nuclear antibodies were detected both in eluates and sera from one seropositive and one seronegative patient Serum antinuclear antibodies were in addition detected in six cases five seropositive and

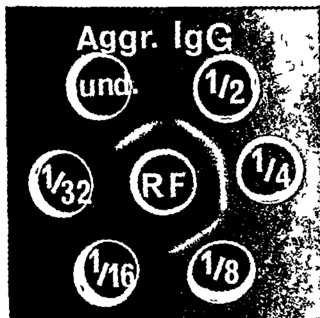


Fig 2 Precipitation in agarose with isolated IgM rheumatoid factor 0 1 mg/ml and dilutions of purified heat aggregates (63°C for 10 min) of IgG 1 5 mg/ml

one seronegative All eluates contained reactant for anti nuclear anti bodies (probably nucleoproteins) which could block these antibodies In the patient providing eluate 141 (Table IV) anti nuclear antibodies appeared in the serum after synovectomies and were weakly present in the eluate

Inhibitor for IgM rheumatoid factor

Inhibitor for IgM rheumatoid factor of the latex fixation inhibition test was found in about 80 % of synovial tissue eluates from seropositive and in 66 % of eluates from seronegative patients It was also found in eluates from rheumatoid nodules and meningeal tissue but not in eluates from control tissues Inhibitor was present in unchanged titer before and after heat inactivation Eluates with high titers of inhibitor precipitated with IgM rheumatoid factor or inhibited the precipitation between IgM rheumatoid factor and heat aggregated IgG (Figs 2 and 3) As previously observed (15) many eluates with high titers of inhibitor showed no free antibodies to IgG regardless of whether these antibodies

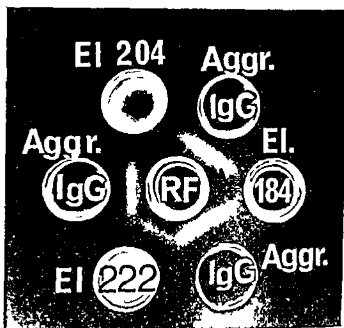


Fig 3 Precipitation and precipitation inhibition with isolated IgM rheumatoid factor 0 1 mg/ml Rheumatoid eluate 222 (IgG conc 0 18 mg/ml) and heat aggregated IgG show a reaction of identity Precipitation inhibition is shown by rheumatoid eluate 201 (IgG conc 0 38 mg/ml) and weakly by eluate 181

were present in high or low titers in corresponding sera (e g eluates 269 65 12 — see table III and fig 5) Eluates without free rheumatoid factor but corresponding to sera with high rheumatoid factor titers invariably contained large amounts of inhibitor (e g eluates 269 [illegible] 440 — fig 5) Eluates without inhibitor and obtained from tissues of seropositive patients had always free rheumatoid factor activity (e g eluates 127 189 176 — table III) with titers varying from 2 to 1 [illegible] Inhibitor was found in some eluates from all variants of seroreactive arthritis but not in eluates from control tissues

Relationship between hidden IgG rheumatoid factor and [illegible] for IgM rheumatoid factor

Results obtained previously (15) showed that a latex fixation activity ascribed to IgG rheumatoid factor was released after pepsin d[illegible] of rheumatoid eluates (Fig 4) Most often there was a good co[illegible] tion between the activity of this IgG rheumatoid factor and the am[illegible]

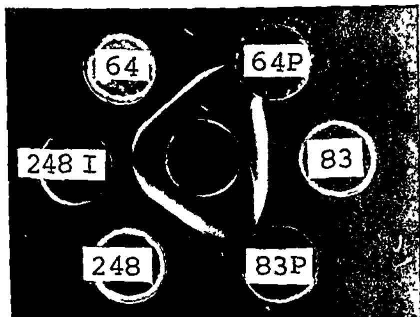


Fig 4 Immunodiffusion test on three eluates before and after pepsin digestion In central well Specific anti IgG Fc fragment P Pepsin digestion with loss of Fc determinants I Incomplete pepsin digestion where Fc determinants are still present

of IgG complexes measured by inhibition in the latex inhibition test (Table III) However some exceptions were noted These were eluates with no or small amounts of inhibitor but still showing high titers of IgG rheumatoid factor after pepsin digestion (e g eluates 127 189 and 176 — table III) Some eluates of seronegative patients such as 141 248 and 35 also gave high IgG rheumatoid factor titers despite showing moderate inhibition of IgM rheumatoid factor Three eluates with retained latex fixation activity after reduction could not be tested for inhibitor and were therefore excluded from the materials However strong latex activity was observed in all three eluates after pepsin digestion

Relationship of IgG complexes to disease activity

Eluates with high titers of inhibitor for IgM rheumatoid factor or of hidden IgG rheumatoid factor were always obtained from strongly inflamed synovial membranes with large masses of granulation tissue

TABLE III

Relationship Between IgG Complexes and Anti IgG Antibodies in Rheumatoid Arthritis

		Eluate no	Total prot conc. (mg/ml)	Titer of RF in serum	Titer of RF in eluate	Titer of PA in serum	Titer of PA in eluate	IgG complexes titrated in eluates by inhibition of IgM RF	IgG complexes titrated in eluates by hidden IgG RF
Seropositive arthritis		269	5 1	512	<1	64	<1	16	16
		65	[illegible]0 3	128	<1	<16	<1	64	64
		12	13 1	32	<1	—	<1	128	3
		177	8 [illegible]	256	128	64	8	<1	3
		189	8 7	512	32	32	[illegible]	<1	3
		176	0 8	10 4	32	128	<1	1	8
		244	3 4	64	4	32	<1	32	64
Seronegative arthritis	a	83	8 [illegible]	<16	<1	16	<1	3	64
		141	4 0	<16	<1	<16	<1		16
		248	12 3	<16	<1	128	4	[illegible]	64
		187	5 1	<16	<1	16	[illegible]	<1	<1
	b	33	8 4	<16	<1	<16	<1		16
	c	71	6 9	<16	<1	<16	<1	2	
	d	64	3 8	<16	2	<16	<1	8	16
		64A	11 7	<16	1	<16	<1	16	4
	e	160	[illegible]8	<16	<1	<16	<1	16	8
Controls		195	[illegible]0	<16	<1	<16	<1	<1	<1
		224	1 3	<16	<1	<16	<1	<1	<1
		230	3 8	<16	<1	<16	<1	<1	<1

RF Rheumatoid factor PA Pepsin agglutinators

Eluates particularly rich in IgG complexes were prepared from granlations taken from the inside of cystic bone erosions On the other hand eluates with few or no indications of IgG complexes were from synovial membranes with low grade inflammation and more fibrous changes (e g eluates 177 155 187 93 and 280 — fig 5) These rheumatoid tissues were from joints with degenerative changes and the patients had low ESR Patients with the highest amounts of IgG complexes had often elevated serum gamma *globulin concentrations* vary from 20 to 40 mg/ml (e g eluates 12 387 293) This was found also

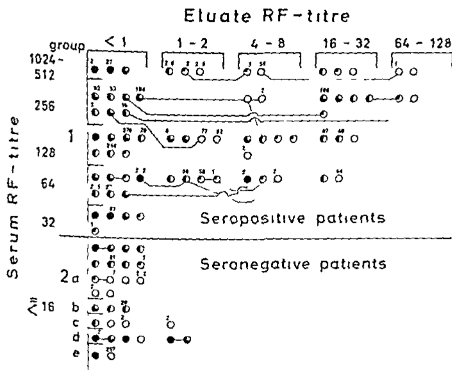


Fig 5 Relationship of inhibitor for IgM rheumatoid factor to free rheumatoid factor activity in eluate and serum Each eluate is indicated by a circle All eluates are from rheumatoid synovial membranes except 440 from the meningeal tissue Inhibition titers of the eluates in the latex test are indicated as follows ● ≧ 16 ◐ 4–8 ◐ 2 ◐ 1 and ○ < 1 The lines connect eluates from tissues of different joints in the same patient Eluates 131 and 256 were from joints injected with osmic acid before synovectomy

in many seronegative patients (e g eluates in horizontal rows 83 — 141 and 161 — 143 — fig 5) Three of these patients had periodically been weakly seropositive The increase in serum gamma globulin was mostly polyclonal

High amounts of IgG complexes were found particularly in three cases of psoriatic arthropathy Two of these patients had severe bilateral knee synovitis which was strongly exudative and erosive (eluates 222 402 and 64 64A) They were diagnosed as cases of psoriatic arthropathy but lacked some of the clinical characteristics such as arthritis in the distal interphalangeal joints Both had definite psoriasis and were

TABLE IV

Titers of Serum Antibodies to IgG Before and After Removal of Complex Containing Synovial Membranes in a Seronegative Patient

Date	Gamma globulin conc (mg/ml)	Waaler Rose test	Latex fixation test	Agglutination of cells coated with anti Rh Ripley	Agglutination of cells coated with [illegible] anti Rh Ripley
2/[illegible] 66	37	<16	<16	—	—
16/11 68	22	<16	<16	<16	<16
25/8 69	18	64	32	128	3[illegible]
18/11 69	—	64	128	16	64
16/1 70	24	128	128*)	32	64
19/1 70	—	64	128	64	64

Synovectomies of knee and finger joints were performed 29/11 68 (eluate 141) and 27/4 69 All serum samples (except from 2/[illegible] 66) were tested in the same series
*) Marked prozone

seronegative However the traces of free IgM rheumatoid factor demonstrated in two eluates (64 and 64A) indicated that IgM rheumatoid factor was produced locally but blocked by the large excess of complexed IgG in the tissue Further evidence for such blocking was obtained in one patient with severe classical RA for 20 years who changed from seronegative to seropositive after removal of synovial membranes containing IgG complexes (Table IV) Her serum had been negative for rheumatoid factor in repeated tests for the last 15 years previous to operations

The findings of IgG complexes and antibodies to IgG were often similar in eluates of different joints in the same patient (e g eluates 104 and 109 from two knees and eluates 160 and 159 from two [illegible] joints — see fig 5) However in some patients eluates from [illegible] contained both rheumatoid factor and antibodies to the pepsin site of IgG

but little or no inhibitor for IgM rheumatoid factor while eluates from another joint showed the reverse pattern (e g eluates 127 and 167 from knee and elbow, and eluates 179 and 198 from two wrist joints)

DISCUSSION

The presence of free IgM rheumatoid factors in rheumatoid tissue eluates is consistent with the immunofluorescent detection of these anti bodies in rheumatoid synovial membranes (10) and subcutaneous nodules (19) Rheumatoid factors have in addition been identified in diseased tissue by mixed agglutination technique (11) One part of the rheumatoid factors in tissue eluates had most likely been produced locally in plasma cells and was perhaps eluted directly from these cells Another part had probably been concentrated in the tissues from sera and joint fluids Eluates from tissues of seronegative patients only rarely contained free IgM rheumatoid factors This could be due to no or minor production or complete blocking of these antibodies The free eluate rheumatoid factors showed no major difference from the serum rheumatoid factors in their reactibility with the different gamma globulin coats However the anti Gm activity found in eluates but not in corresponding sera suggested that some rheumatoid factors were preferentially bound in the tissues

Antibodies to the pepsin site of IgG are mainly of IgG type (see 15 17) They have been detected in a variety of disease states and also among healthy individuals Patients with rheumatoid diseases often have these antibodies in their sera They are frequently found in seropositive RA but also in seronegative cases for example juvenile RA (12) Such antibodies can be detected in rheumatoid tissue by immunofluorescent technique (13 14) and their presence in rheumatoid tissue eluates further confirms their concentration in diseased tissue

Comparative studies of joint fluid and serum titers of antibodies to IgG have indicated local production and blocking of both IgM rheumatoid factors (3) and antibodies to the pepsin site of IgG (9) in rheumatoid joints Some of the blocked antibodies are possibly deposited in the tissue In contrast to naturally occurring IgM and IgG serum antibodies they are only partially removed by extensive washing of the tissue

Complexes of IgG with complement have been identified in rheumatoid synovial membranes and isolated synovial lining cells by immunofluorescent technique (1 5 13 14) The IgG complexes present in most rheumatoid eluates could be identical to those seen in tissue sections Such complexes probably act as antigens and reactants for antibodies to IgG The striking observation in the present study was the *lack of free IgM rheumatoid factor in 41 % of eluates from synovial tissues* of seropositive RA and the ease with which IgG complexes in these eluates were demonstrated Furthermore prozones were found in eluates with free antibodies to IgG in addition to IgG complexes This indicated that the antibodies could be blocked by IgG complexes The complexes reacted with and in some cases precipitated IgM rheumatoid factor in vitro This may also occur in vivo In eluates from both seropositive and seronegative patients IgG rheumatoid factor was revealed after pepsin digestion indicating that this antibody was produced in *all variants of RA*

Failure of some rheumatoid eluates to inhibit the IgM rheumatoid factor used in latex inhibition test could possibly be due to in vivo saturation of IgG complexes with that type of antibody Free IgM rheumatoid factor was often present in such eluates indicating antibody excess Although the eluates were reduced and alkylated before being tested this procedure does not destroy the blocking capacity of the subunits of IgM rheumatoid factor (2 22 24)

The amount of IgG complexes could also be expressed by the IgG rheumatoid factor activity obtained after pepsin digestion, assuming that IgG rheumatoid factor constitutes a major part of the complexes Pepsin digestion does not abolish the agglutinating activity of IgG antibodies but destroys the latex precipitation by IgM rheumatoid factor (4) *The latex precipitation activity released after pepsin digestion* was therefore ascribed to IgG antibodies However degradation of IgM rheumatoid factors by pepsin also provides active fragments able to react but not to precipitate with altered human IgG (23)

Other investigations have shown that IgG rheumatoid factors in serum are bound to autologous IgG in 7 to 19 S *intermediate complexes* (7 21) Such complexes had latex precipitation activity (21) in contrast to most of the unsplit eluate IgG complexes This indicates that IgG rheumatoid factor has a higher avidity *for IgG in the* rheumatoid tissue than *for IgG in the serum*

The antigen which induces IgG rheumatoid *factor* formation may

be IgG in immune complex or an otherwise altered IgG The antibodies to IgG were eluted together with their antigens but some IgG com plexes were possibly left in the tissue Other uncharacterized antigens for IgG antibodies may also have evaded elution

The present findings are consistent with the hypothesis that IgG complexes are pathogenetic factors in rheumatoid arthritis They were most clearly demonstrated in eluates from highly inflammatory tissues When different joints in a patient showed the same degree of arthritis IgG complexes were present in equal amounts in the respective eluates IgG complexes in the affected joints seemed to influence the serum titer of IgM rheumatoid factor Thus IgM rheumatoid factor could evade detection in some seronegative patients by fixation to IgG complexes

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Kass Erik Acta Rheum Scand 16 257—260 1970

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COLLABORATION BETWEEN RHEUMATOLOGIST AND ORTHOPEDIC SURGEON — A NECESSITY

By

ERIK KASS

The importance of orthopedic surgery as an auxiliary to any treatment of rheumatoid arthritis is underlined in the present article No treatment programme for rheumatoid arthritis is satisfactory if it does not recognize this fact It must form part of all future planning for hospital care of rheumatological patients

Patients with rheumatic diseases must have a combined treatment programme Although surgical therapy is often very important and in some cases doubtless the most important form of treatment it can not be regarded isolated from the other forms The main reason that orthopedic surgery did not come into its own earlier was the insufficient recognition of this basic fact and the consequent disappointments

An effective teamwork is necessary and this is only possible when all members of the team daily and without difficulty can have contact with one another and with the patients It must be emphasized that one is dealing with chronic sufferings which in many cases occasion a high degree of invalidity and which often result in serious problems and psychic difficulties for the patients Because of his disability a patient is often socially isolated to an alarming degree and will have difficulty in making contacts while at the same time his need for contact is very pronounced On purely humanitarian grounds it is therefore necessary that during the whole of his hospitalization the patient is allowed to retain the contact with and feeling of confidence in the doctors and the permanent nursing staff which has presumably been established

In connection with the problems raised here the following quotation from the Technical Conference on the Public Health Aspects of Chronic Rheumatoid Arthritis and Related Diseases, held in Rome during 1963, may be of interest A very important requirement is continuity of treatment since the best results are obtained by carefully planned *regimen extending* over the various phases of the disease Sudden changes in the regimen which often occur from the care of one specialist to another, or when he is admitted to other hospitals for intercurrent diseases may have adverse and sometimes disastrous effects on the treatment of the rheumatoid disease The Oxford orthopedic surgeon Geoffrey Platt writes (Hill A G S Modern Trends in Rheumatology London Butterworths 1966) Where special wards are available for rheumatological cases it is advantageous to carry out the surgical treatment without transferring the patient elsewhere, thus ensuring continuity of medical treatment The orthopedic surgeons Osmund Clarke and Michael Mason write in the latest edition of Copeman s textbook The Orthopedic and the Rheumatology Departments *should ideally be close together i e they should be housed* in the same building on the same floor using the same administrative service for appointments and record keeping Experience from Oslo Sanitets forening s Rheumatism Hospital can also illustrate the importance of this It is obvious that collaboration between the rheumatologist and *the orthopedic surgeon is a necessary precondition* The first serious expansion in orthopedic surgery at Oslo Sanitetsforening s Rheumatism Hospital took place in 1968 when as many as 515 operations were performed A total of 957 patients were hospitalized during this year The figures for 1969 were 630 and 928 respectively Increasingly differentiated operations are performed

There is however one thing which must not be forgotten This development has been and still is centred around the Rheumatism Foundation Hospital in Heinola The initial spadework was done by Veikko Laine and Kauko Vainio and *the significance of their contribution* cannot be overestimated *Orthopedic operations* of various kinds both synovectomies and reconstructive surgery of various types had of course been performed during previous years but it is especially because of the Finnish pioneer work that attention has been drawn to the significance of surgery in the combined treatment programme and for this reason there must be a *surgeon on the team of doctors* employed in the special departments for rheumatological patients In

those departments, where such collaboration actually is introduced it is shown that 50—70 % of the patients — and the percentage may in reality be higher — are in need of one or more surgical operations The problems associated with R A surgery are different from those applying for osteoarthritis surgery, surgery for poliomyelitis sequelae cerebral paralysis or after effects of injury This applies not only to the operation itself but also to the pre treatment and post treatment

Not only is the collaboration outlined quite decisive for the treatment of patients it is also of prime importance for the eventual instruction of students and all medical and paramedical personnel as well as for doctors in educational positions and for a number of research projects

The rheumatic diseases are on the whole very exacting both diagnostically and as far as treatment is concerned The patients can show symptoms from a variety of organs The entire medical apparatus must be employed and collaboration between specialists in the most varying medical branches is required If this is to be done in a satisfactory manner the Rheumatology Department must be part of a fully equipped and comprehensive Regional Hospital but there is the absolute condition that this Regional Hospital does possess a specialist department for rheumatological cases Otherwise the treatment can never be satisfactory During recent years medicine has become more and more specialized and the consequences of this must be accepted in all hospital policy Developments in treatment methods have made the placing of our rheumatological patients in non specialized departments less and less rational It is furthermore of the utmost importance that the patient can be given a combined treatment at one and the same place

The different kinds of handicap make differentiated treatment necessary At the same time it is rational to make full use of the common treatment possibilities as far as possible This is one of the reasons that orthopedic surgical rheumatological and neurological departments should be planned simultaneously within the Regional Hospital These departments supplement one another with regard to handicapped patients The disease groups are closely related and an intimate cooperation is necessary to achieve the best possible therapeutic results The physical/medical department can be of service for the departments mentioned above and naturally for the other departments of the hospital as well A department for rehabilitation will also be of importance but the first step in the development must be that the patient has

dergone examination and adequate treatment in the other specialist departments — which therefore *must* already have been established if the rehabilitation department is really to be fully used to the patients full advantage

Planning must be viewed collectively There must be no discussion as to whether there should be a department for rheumatology neurol ogy orthopedic surgery physical medicine or rehabilitation All these departments represent in reality a unit with very many mutual tasks tasks which are *nevertheless so dissimilar that there is no* question of one department automatically taking over the functions of another It must be emphasized that the orthopedic surgeon must in all instances be incorporated in the rheumatology team This is an absolute necessity

My main conclusion must be that the advance of orthopedic sur gery represents the most important progress within the treatment of rheumatoid arthritis during recent years Consequently no treatment programme is satisfactory which does not recognize this fact which must therefore also play a decisive role in all future planning for the hospital care of rheumatological patients

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DISPERSED CELL CULTURES OF RHEUMATOID SYNOVIAL MEMBRANE

By

D G PALMER

Summary The morphology of cells released from synovial tissues involved by rheumatoid arthritis and grown in tissue culture has been described Cultures were well established and differentiated by the third day In growth medium enriched with heat inactivated rheumatoid serum syncytial cell masses frequently formed This change particularly was very like the characteristic cytopathic effect of a number of viruses

Cell cultures established from explants of human synovial membrane have been used to study the growth metabolism and morphology of cells derived from both normal synovium and from synovium involved by rheumatoid arthritis (3 7 8 10)

In the present study cultures were established from those varied cell forms which could be released by the action of trypsin on surgical specimens of rheumatoid synovium For studies of a tissue as a whole this technique has certain advantages over the explant method All cell forms in the original tissue have the opportunity to develop in the culture system growth is rapidly established and the cultures lend themselves to further manipulation

MATERIALS AND METHODS

Excised synovium was obtained at the time of operation from thirty five joints or tendon sheaths involved by RA The donor patients

had erosive RA and with one exception were seropositive for rheumatoid factor Patients receiving corticosteroid therapy received booster doses of hydrocortisone prior to surgery The synovium was snipped from the overlying capsule and the fragments were incubated and agitated for 45 min at 37°C in a 0 25 % solution of trypsin*

Cells freed by this treatment were separated from tissue debris by filtering through cotton gauze centrifuged for 10 minutes at 1 000 rpm and resuspended at a concentration of 5 000—6 000 cells/cmm in serum free (21) medium 199 Four to six drops of the suspension were distributed on to each of a number of cover slips seated within sterile disposable 50 mm Petri dishes After 1/2—1 hour 4 5 ml of medium 199 containing 20 % of a chosen serum was added before incubation in an atmosphere of 5 % carbon dioxide in air at 37 C

Various sera were used in the experiments namely fetal calf serum autologous sera from donor patients undergoing synovectomy homologous sera from other rheumatoid patients and homologous sera from normal persons Sera simply separated from the spontaneous clot have been referred to as untreated Sera heat treated for 30 min in a water bath at 56 C have been referred to as inactivated

Media were changed at 2 or 3 day intervals Cover slips were removed periodically fixed in 10 % formol saline and stained with either haemalum and eosin or with May Grunwald Giemsa before examination by light microscopy An examination of a cell culture under phase using timelapse cinematography was undertaken

RESULTS

Nucleated Cell Forms Released by Trypsin

Cells found in suspension after digestion of rheumatoid synovial tissues consisted of small mononuclear cells (mainly lymphocytes and plasma cells) large mononuclear cells (mainly macrophages and synoviocytes) and occasional polymorphonuclear leukocytes Other cell types such as fibroblasts and capillary enothelial cells were no doubt present Table I sets out the ranges and average distribution of these three cell classes in differential counts from twenty two suspensions

Cultures were well established within 72 hours if the initial cell concentration was between 5 000 and 6 000 cells per cmm and if four to six drops of such a suspension from a Pasteur pipette were placed on each cover slip

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* Difco Laboratories prepared in phosphate buffered saline

TABLE I

Distribution of Cell Forms Released by Trypsin from 22 Specimens of Rheumatoid Synovium

Cell form	Range %	Mean %
Small mononuclear cells	11—60	39
Large mononuclear cells	26—88	55
Polymorphonuclear cells	0—22	6

The Morphology of Rheumatoid Synovial Tissue Cells Grown in Media Containing 20 % Fetal Calf Serum

(a) *Fusiform and stellate cells* These cells which were probably of fibroblastic origin were present in all cultures but were not necessarily the dominant cell They contained little cytoplasm and had a small dark nucleus with 1—3 nucleoli Small granules were present throughout the cytoplasm

(b) *Lymphocytes plasma cells granulocytes and macrophages* Taken together the lymphocytes plasma cells and granulocytes made up about 50 % of the cell population at the third day but thereafter these cells (excepting the macrophages) diminished in number in part due to loss with changes of culture medium

The macrophage appeared either as a large round cell with a centrally placed nucleus containing 1—3 nucleoli or as an elongated cell with two or more processes terminating in expanded pseudopodia The perinuclear cytoplasm was granular and heavily stained A stout process (uropod) was sometimes seen projecting from this central perinuclear area The cinematographic study showed these processes to be successively protruded and withdrawn Intercellular links were formed on successful contact with another cell

(c) *Multipolar cells* These cells were broadly invested by homogeneous cytoplasm which was prolonged at two or more points into tapering processes Their nuclei were large stained lightly and contained 1—3 large distinct nucleoli Fragmentation of one or more of these nucleoli was common Proliferation of these cells led to the progressive formation of irregular multilayered cellular systems (Fig 1)

(d) *Multinucleated cells* Cells with at least six nuclei were f in most cultures Although such cells seemed to develop most



Fig 1 Multipolar cells have proliferated to form a dense irregular multilayer (Untreated autologous serum 7 day culture H and E stain x 190)

from the macrophage type cells (Fig 2) they occasionally formed from the multipolar cells for the characteristics of either one or other form could still be recognised at an early stage of development Cells with up to nine nuclei could be found within 24 hours of establishing cultures and

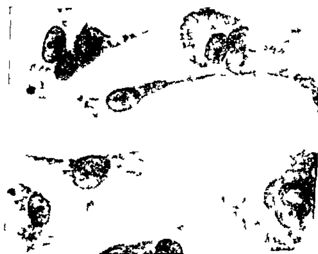


Fig 2 Macrophage type cells showing early stages of multinucleation (Fetal calf serum 7 day culture May Grunwald Giemsa stain x 1 200)



Fig 3 Polykaryocyte formation in the presence of untreated autologous serum Cell clumps (arrows) are present (3 day culture H and E stain x 190)

by the third day cells containing around 80 nuclei were frequent Significant further change did not occur in these cells when followed for as long as ten days With increasing cell size the multiple nuclei became smaller but one to three nucleoli in each nucleus remained visible

Cell Morphology in the Presence of Untreated Human Serum

Cultures grown in medium containing untreated autologous rheumatoid serum homologous rheumatoid serum or homologous normal serum developed rather similarly to cultures grown in fetal calf serum including the appearance of multinucleated cells Macrophage like cells however tended to be rather more conspicuous than the multipolar forms Cell clumping was common in the presence of such untreated sera (Fig 3)

Cell Morphology in the Presence of Inactivated Human Serum

Inactivated rheumatoid sera (both autologous and homologous) produced differences in cell growth in all of seven cultures when compared with growth in the presence of the corresponding untreated sera Macrophage like cells were again the dominant cell but multinucleated cell formation was greatly accelerated and large irregular syncytial mas frequently containing 200—500 nuclei appeared by the third

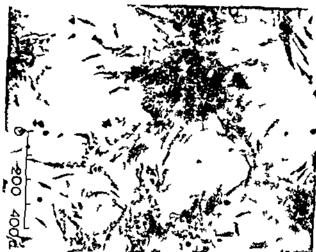


Fig 4 Syncytial mass formation in the presence of inactivated autologous serum. (3 day culture H and E stain x 100)

culture (Figs 4 & 5) The remaining mononuclear macrophages and smaller multinucleated cells showed a marked tendency to direct a central process into the perinuclear zones of these large syncytial masses into other macrophages and into clusters of round cells (Fig 6) Nucle

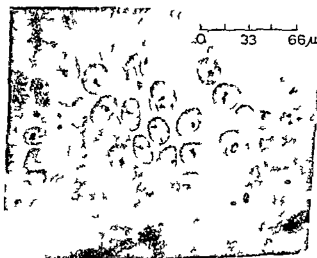


Fig 5 Detail of nuclei from the syncytial mass shown in fig 4 Nucleolar irregularity is apparent (3 day culture H and E stain x 1 00)



Fig 6 Linkages between two polykaryocytes and the periphery of a syncytial mass Note that these processes arise from the central granular perinuclear cytoplasm (Inactivated autologous serum 3 day culture H and E stain x 576)

were occasionally seen in such processes Aggregation and peripolesis of round cells on multinucleated cells was observed by cinematography

DISCUSSION

Although altered cellular activity may result from exposure to trypsin (13 14 17 24) the morphology of the cells described in this study apart from the syncytial masses seemed closely similar to that of cells migrating from explants of synovium (3 30) It was possible to recognize the probable origins of the various cells present in these cultures particularly the *lymphocytic granulocytic* and *fibroblastic* forms The macrophage type cells were most probably derived from tissue histiocytes The *multipolar cells* may have been of fibroblastic origin (30) but as similar cells are to be seen in cultures established by the technique of ...nt trypsinization (12) such cells may have been derived from synovio...tes

The marked clumping of cells noted in a number of cultures in the presence of untreated human serum may have reflected the clumping effect possessed by some human sera (26) The possibility of this phenomenon being of the nature of immune adherence or of an infective ...opathic effect was not excluded

Particular physical relationships between macrophages and lymphocytes in culture such as peripolesis (27) rosette formation (4) and cell linkages (2 5 16) have been thought to reflect immunological activity Adherence of lymphocytes to synovial fibroblasts however is not restricted to cells of rheumatoid origin (29)

Multinucleated cells have been noted to form in cultures of various cell strains if the pH of the culture medium is low (24), if the serum in the medium is of bovine origin (20) after exposure to radiation (32) or if certain viruses are present in the cell culture In the present series of experiments multinucleated cell formation was independent of pH change of bovine serum and of radiation exposure

Multinucleated cell formation both in vivo and in vitro is a well recognised cytological response to a variety of viruses and may result from either abnormal mitosis (25) or cell fusion (23) Macrophages in particular may selectively harbour certain viruses (18 19) and may become multinucleated (1) In vivo multinucleated cell formation is a common cellular response to various disease processes including both the synovial response (10 15) and the pleural reaction (22) of rheumatoid disease The polykaryocyte and syncytial mass formation reported in these dispersion cultures of rheumatoid cells could possibly represent a cytopathic response to the presence of some as yet unrecognized infective agent Reported attempts to identify a virus in subcultures of fibroblast type cells derived from rheumatoid synovium however have not as yet been successful (9 11 33) although some indirect evidence for such a possibility has been put forward (28)

Multinucleated cells (but not syncytial masses) have been observed amongst cells migrating from explants of normal as well as rheumatoid synovial tissues (3 30) and normal monocytes may with time give rise to multi nucleated cells in culture (6 33) In the present experiments however the *early* appearance of large syncytial masses (in the presence of inactivated rheumatoid serum) rather indicated a distinctive phenomenon If the activation of a virus was responsible for this change both h at inactivation of complement and destruction of interferon by trypsin in the process of establishing cultures may have been of importance

In this study normal contact inhibition between cells appeared to be lost in some cultures with overgrowth of the multipolar cells to produce dense multilayered systems Growth patterns of this nature may also follow viral inoculation of a cell culture (31)

Dispersed cell cultures of synovial membranes should prove useful in the investigation of rheumatoid arthritis and should be adaptable to further morphological immunological and virological studies

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ADDENDUM

Since submitting this paper for publication three papers have appeared which are of importance in considering the likelihood of RA being due to a viral agent These are

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TECHNETIUM 99m IN THE STUDY OF RHEUMATIC JOINTS*

By

MARTTI OKA AHTI REKONEN and AHTI RUOTSI

Summary The accumulation of Tc 99m in knee and finger joints was studied in 41 rheumatic patients and 25 controls The accumulation of the radioisotope was correlated with the intensity of active inflammation Rheumatoid joints showed a stronger Tc 99m uptake than joints with osteoarthritis There was some overlap however, which limits the value of the method The effect of local and systemic anti rheumatic treatments could be followed with joint scanning

Radioactivity was also measured in the synovial fluid but this fraction of the Tc 99m was not large The synovial fluid radioactivity tended to increase as the Tc 99m accumulation in the knee area decreased Accumulation of Tc 99m in joints is regarded as principally due to the enhanced circulation in inflamed joint tissues

The assessment of inflammatory activity in rheumatic joints is often difficult Reliable objective methods are lacking Clinical symptoms of inflammation (elevated temperature redness) are usually slight or absent Swelling and pain on the other hand are not direct parameters of inflammation Valuable information can be obtained by analysis of synovial fluid but this is not always possible X ray changes in the joint structures show the degree of damage produced by the disease process but not the actual degree of inflammatory activity

* Preliminary report read at the XII Scandinavian Congress of Rheumatology Helsinki, June 1 1968

Alarcon Segovia and co workers (1) adopted joint scintigraphy with the aid of intravenously injected technetium 99m (Tc 99m) for the study of joints They found that uptake of the isotope by inflamed joints was higher than by normal or degenerated joints Synovial biopsies examined showed no significant uptake of Tc 99m but moderate radioactivity was observed in synovial fluid In rheumatoid joints Whaley et al (4) found a marked uptake of *intravenously injected Tc 99m* but almost no uptake of radioisotope by normal joints The isotope was not detected in the diseased synovial membrane Maxfield et al (3) demonstrated that scintillation scanning of the joints is a sensitive method for evaluating an articular process In their view the localization of the isotope is due to increased permeability of the synovial membrane rather than to increased vascularity

In the present investigation we have used the radioactive isotope Tc 99m in the study of inflammatory activity in the knee and finger joints The effect of local and systemic anti inflammatory agents has also been estimated with this method The mechanism of Tc 99m accumulation *in the joint as well as the value of joint scanning in the differential* diagnosis of arthritis has been studied

MATERIAL AND METHODS

The series comprised 41 patients with the following joint diseases 27 with rheumatoid arthritis 1 with Reiter s disease 1 with ankylosing spondylitis 2 with postinfectious arthritis 1 with intermittent hydrarthrosis 9 with osteoarthritis

In this series there were 10 males and 31 females with ages ranging from 19 to 71 years (mean 49 years) The control group comprised 25 patients without joint diseases These received a dose of Tc 99m (10 mCi) for brain scanning

The tracer used in the study was the radioactive isotope Tc 99m in the form of sodium pertechnetate The radiophysical characteristics of Tc 99m are as follows It emits gamma radiation of energy 140 keV and its half life is 6 hours The whole body radiation load is around 100 mrad with the dose used

10 mCi of Tc 99m was injected intravenously 30 minutes before the study Scanning was performed with a Picker Magnascanner III using a low energy collimator Some of the pictures were taken with a

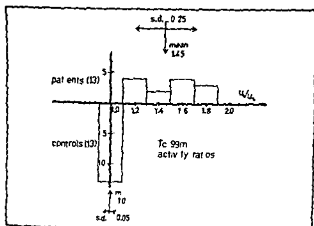


Fig 1 Tc 99m activity ratios between affected and unaffected knees in RA patients compared with the ratios in control cases Patients U_1 = uptake in affected joint U_2 = uptake in unaffected joint Controls U_1 = uptake in left knee U_2 = uptake in right knee

gamma camera (Pho Gamma III Nuclear Chicago) The number of knees scanned was 88 and of hands 12 The uptake of Tc 99m in the joint area was measured in 13 patients and 13 controls 30 minutes after the injection using a conventional J 131 uptake counting apparatus The measurements were performed in both the affected and the contralateral unaffected knee

The radioactivity concentration in synovial fluid and serum was measured in 14 patients The samples were taken 30 minutes after injection and followed in two cases for up to one hour The accumulation of Tc 99m in the knee joints was continuously measured for about one hour in 12 patients The apparatus used was a conventional renography system

In 12 cases repeated Tc 99m scannings were performed after local or systemic treatment as follows 5 cases after local injection of hydrocortisone acetate 1 case after systemic therapy with large doses of prednisolone 5 cases after local osmic acid injection 1 case after evacuation of the joint and washing with physiologic saline

A pilot study was performed in two cases about Tc 99m concentration in synovial membrane specimens taken during knee joint synovectomy

Inflammatory activity and Tc 99m accumulation were estimated by different persons independently

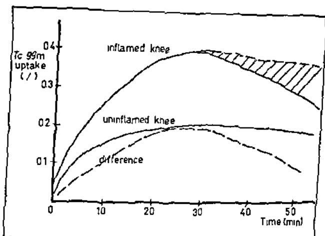


Fig 2 Typical Tc 99m accumulation curves of inflamed and uninflamed rheumatoid knee joints The shaded area indicates the individual variation in the descending part of the curves of the affected knees

RESULTS

The externally measured Tc 99m uptake values are shown in fig 1 The uptake values are presented as activity ratios between inflamed and uninflamed joints of the same patient In the control series the comparison is presented as left/right ratios There was a highly significant statistical difference ($p \leqslant 0.001$) in the activity ratios between the joint disease group and the control series

Typical Tc 99m accumulation curves are presented in fig 2 The rise of the curve is clearly steeper in the affected knee than on the contralateral side The maximum of the curve varies between 10 and 41 minutes and the difference between the normal curve and that for the affected knee is usually greatest about 30 minutes after injection

The results of the radioactivity measurements from the joint fluids are presented in the table Figures are presented as concentration ratios between synovial fluid and serum It seems that the ratio is near 1 in mildly inflamed knees The ratio is smaller in the knees with more severe inflammation The synovial fluid radioactivity tended to increase as the Tc 99m accumulation in the knee area decreased The total joint fluid Tc 99m activity did not exceed 20 per cent of the total radioactivity in the knee area

TABLE I

The Results of the Radioactivity Measurements in Joint Fluid Presented as Concentration Ratios between Synovial Fluid and Serum

Inflammatory activity of the joint	Tc 99m concentration joint fluid/serum	Mean
1	0 90	0 69
	0 80	
	0 76	
	0 79	
2	0 47	0 38
	0 45	
	0 27	
	0 25	
3	0 60	0 [illegible]9
	0 46	
	0 22	
	0 21	
	0 20	
	0 15	

The samples were drawn 30 minutes after Tc 99m injection Degrees See legend to fig 3

Fig 3 shows the scanning results A comparison is made between the estimated Tc 99m accumulation and the clinically evaluated inflammatory activity These results are only qualitative in character but it is clearly seen that Tc 99m accumulation follows the clinical inflammatory activity of the joint

Tc 99m accumulation was increased in those finger joints in which signs of inflammation were clear but there was also detectable Tc 99m accumulation in some joints where a clinical observer could not detect any sign of inflammation

Fig 4 shows a typical scanning picture of knee joints with asymmetric involvement

Fig 5 presents the effect of treatment with intra articular hydrocortisone acetate or osmic acid and oral prednisolone on estimated Tc 99m accumulation and clinical activity Both were diminished after the treatments

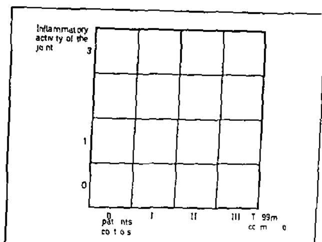


Fig 3 Clinical inflammatory activity of the joint compared to Tc 99m joint scans in RA patients and healthy controls Inflammatory activity 0 no symptoms 1 pain on motion periarticular swelling 2 — pain and hydrops 3 = large effusion and elevated temperature Tc 99m accumulation 0 — no accumulation I = slight diffuse accumulation II = moderate accumulation joint structure visible III = strong accumulation joint structure clear

There was no appreciable change in the scanning pictures taken before and after removal of synovial fluid from the joint cavity 30 minutes after injection of Tc 99m



Fig 4 Typical scanning picture of knee joints with asymmetric involvement

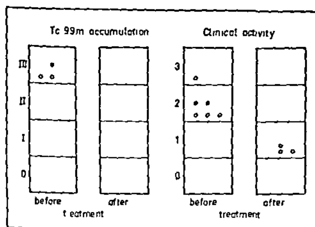


Fig 5 The effect of various treatments on Tc 99m accumulation and clinical inflammatory activity in rheumatoid knee joints
+ oral prednisolone administration
o intra articular injection of hydrocortisone acetate
• intra articular injection of osmic acid
Degrees See legend to fig 3

In the two synovial tissue specimens taken during knee joint surgery there was detectable Tc 99m accumulation in both

In the osteoarthritic joints studied there was no Tc 99m accumulation in 4 cases slight accumulation in 10 and moderate accumulation in 4

DISCUSSION

The data presented in this paper clearly show that Tc 99m scanning can be used with success in the study of joint diseases The accumulation of the radioisotope in the joint was clearly correlated with the intensity of active inflammation In joints with clinically active RA the accumulation of Tc 99m was stronger than in normal joints In RA the accumulation of the isotope was more pronounced in the joints in which the clinical activity was most severe After treatment with local anti inflammatory agents (hydrocortisone osmic acid) the joint scans showed less marked uptake of radioactive material than before treatment The situation was the same after oral administration of large doses of steroids

Accumulation of Tc 99m in joints with osteoarthritis seemed to be greater than in the control joints and generally less great than in rheumatoid joints However there were some cases which could not be differentiated from RA by this method Thus the value of Tc 99m joint scanning in the differential diagnostics of joint diseases seems to be limited The main indication of joint scanning in clinical practice is to demonstrate and measure active inflammation

It is obvious that in the knee joint an uptake measurement will give a better measure of the degree of Tc 99m accumulation than the scanning technique But the information obtained by joint scanning is usually sufficient for clinical purposes The sensitivity of the method is greater in small joints Scintillation scanning of joints is a simple and rapid method especially if a gamma camera is available

The accumulation of Tc 99m in joints seems to be a reflection of enhanced circulation in the joint tissues Radioactivity can also be detected in the synovial fluid but this fraction of Tc 99m is not large It was further observed that there was less radioactivity in the synovial fluid in the cases with intensive Tc 99m accumulation in the joint area than in those in which uptake of the isotope was slight or moderate

Some previous workers (1 4) have been unable to observe active uptake of the isotope by the synovial membrane The synovial biopsies studied by us after intravenous administration of Tc 99m showed that the isotope is actively taken up by the synovial membrane

In some RA cases the rate of disappearance of Tc 99m from the joint slowed down after about half an hour This difference may have been due to changes in the capillary arteriovenous circulation in the synovial membrane of RA patients (2) The delayed slope of the curve may also result from retention of Tc 99m in the synovial fluid

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ELECTRODIAGNOSTIC INVESTIGATION OF THE NEURO MUSCULAR LESIONS IN RHEUMATOID ARTHRITIS

By

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Summary 85 muscles, in 30 rheumatoid arthritis patients were examined by EMG The patients were subdivided into steroid and non steroid *treated groups*

In both groups, the EMG demonstrated myopathic, polymyositic and neuropathic lesions the first two predominating Similar changes were found regardless of the size of the muscle examined A relatively high number of myopathic changes were observed in early RA The activity of the disease did not seem to play an important role *No correlation* with the condition of the adjacent joint was found No patient had a normal EMG in all the muscles examined The conduction velocity indicated an entrapment syndrome in four patients Creatine phosphokinase was normal in 23 patients while aldolase was moderately high in 9 of 23 cases

The muscle and peripheral nerve may be involved in any stage of rheumatoid arthritis (RA) *Typical histological lesions have been de*scribed in both muscles (7 10 17, *21 25 27 38 40*) *Similar* lesions and peripheral nerves (15 19 20)

The *presence* of electromyographic (EMG) evidence of myopathic lesions has often been reported (1 2 *6 8 9 14 22 26 30 33 36* 37, 39, 41 42 45 46) The criteria for such an interpretation of the EMG recording have not always been clearly defined and *furthermore*

the relation between the damaged muscle and the condition of the adja cent joint has sometimes not been mentioned Neuropathic lesions have also been described but the evidence seems controversial Additional confusion has been caused by the failure always to distinguish between RA patients receiving steroid therapy and those who were not Since the reports of Antopol (3) and Ellis (12 13) it has been known that cortisone may cause widespread necrosis of the muscle with signs of regeneration (13 43) Myopathy has also been observed with all the steroids (3—5, 8 12—14 18 22 23 32 37 42 43) but mainly with those containing fluor in position 9 (1 11 16 24 28, 29 41 44 45) Only few conduction velocity (CV) tests have been carried out (2 6 14 26 30 39)

MATERIALS METHOD AND TECHNIQUE

The basic studies were carried out in 30 patients who according to the classification of the American Rheumatism Association (34 35) had definite RA Of these 30 patients 14 had been receiving steroid therapy mainly prednisone flumethone or dexamethasone for a period of at least six months prior to our investigation This group aged be tween 37 to 66 years consisted of 13 women and one man The second group of 12 women and 4 men aged between 35 to 64 years had never as far as we could ascertain received any steroids and had been treated with indomethacin salicylates gold salts or phenylbutazone The activ ity of the disease was assessed according to the clinical signs of in flammation ESR and C reactive protein Five patients in the steroid group were classified as active and 9 as inactive The corresponding figures in the non steroid group were 5 and 11 respectively The dura tion of disease judged from the onset of the first complaints was from 1 to 3 years in 12 patients 4—6 years in seven 7—10 years in six and more than 11 years in five patients in approximately equal numbers in each of the two groups

The 30 patients underwent a thorough general and neurological exa mination special care being paid to the presence of atrophy loss of motor power Tinel sign and the condition of the adjacent joints The joints were classified into four basic groups according to their clinical condition

Normal — full range of motion completely free of pain no loca lized signs of inflammation and in normal daily use

TABLE I

Relation between Muscles Examined by EMG and Condition of Adjacent Joints

	Steroid group joint damage				Non steroid group joint damage				
	Normal	Mild	Moderate	Severe	Normal	Mild	Moderate	Severe	Total
Small muscles									
Ist Dorsal Interosseus	1	2	5	6	—	4	6	5	29
3rd Palmar Interosseus	1	2	5	6	—	2	6	3	5
Abductor digiti minimi	—	—	1	1	—	—	—	—	
Large muscles									
Quadriceps	—	—	3	5	1	1	2	2	14
Medial deltoid	1	1	1	—	2	—	—	—	5
Biceps humeri	—	—	1	2	—	—	—	—	3
Brachio radialis	—	—	—	2	1	—	—	—	3
Upper trapezius	1	1	—	—	—	—	—	—	2
Anterior tibial	2	—	—	—	—	—	—	—	2
Total	6	6	16	22	4	7	14	10	85

Total small muscles 56
Total large muscles 29
Total of muscles examined in steroid group 50
Total of muscles examined in non steroid group 35

Mildly damaged — full range of motion pain only in the extreme range of motion with or without mild local inflammation and in normal or almost normal daily use

Moderately damaged — limitation of motion with pain upon movement of the joint and limited use

Severely damaged — severe or complete ankylosis and very limited or no use of the joint

Electromyography (EMG) was carried out in several muscles from each patient The examined muscles were selected according to their strength atrophy and location The EMG investigation consisted of the usual visual analysis of the action potentials and in order to corroborate the results duration peak to peak and interval histograms of the spike Only teflon coated coaxial needle electrodes were used These were connected to an 8 channel dynamic recorder with a potential amplification

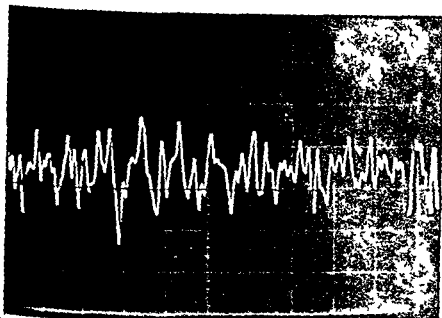


Fig 1 Photograph of EMG from the 1st dorsal interosseus muscle during maximal volitional contraction Calibration 2 5 m sec and 150 micro V per square Decreased duration low amplitude multi peaked spikes and a full interference pattern are noted

of x20 000 provided with an 8-channel ultraviolet unit This recorder was linked through a 7 + 1 high sensitivity FM tape recorder to a computer of average transients (CAT) Visual display was on a 4-channel oscilloscope with memory storage Audio monitoring was made with an industrial loudspeaker EMG was carried out on a total of 85 muscles 56 of them were small intrinsic muscles of the hand and 29 were large muscles of the limbs (Table I) 50 muscles were examined in the group of patients receiving steroids and 35 in the non steroid group The adjacent joints were normal in 6 steroid and 4 non steroid patients mildly damaged in 6 and 7 respective groups moderately damaged in 16 and 14 and severely damaged in 22 and 10 in the steroid and non steroid groups respectively

Interpretation of the EMG pattern as myopathic was based on decreased duration of the action potential or spike tendency to fragmentation of the motor unit decreased amplitude the possible presence of polyphasic potentials with brief duration and a full interference pattern (F

F g Ph t g aph t EMG fr m med al del o d muscle during maximal mu t nal nt a t n Ca b a n m e and 150 m ro V per square Decreased durat n l w an pl ude f agmen ed p ke and polyphas c potentials with bre du a n ma be b er ed

1) A neur path c EMG pattern was man fest by loss of motor u s pr longed durat t p tent als p lyphas c potent als w th increased duration and met me spontane us acti ity (Figs 3 4) In s m mus les both my path a d neur path EMG patterns were preser but without h gh nsert onal act v ty these record ngs were d agnosed as polymyos t s The EMG d agnos s was made only after the needle was relocated at lea t tw e n ea h nd vidual mu le If as happened in a few nstan e ea h loc t n of the needle electrode showed eviden e f a d ff nt t pe t pattern the record ng was nterpreted as polymyos t s

In 15 patients f n th s ser es 8 in the stero d and 7 in the non steroid group a 1 nd cti n velocity test (CV) was carried ou on the ulnar nerve an l on the mmon peroneal nerve The stim l s was given by a squa p l e st mulator with ground isolat on the dura tion of stimulus was ond and the frequency one every two seconds

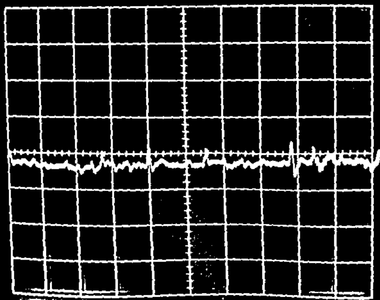


Fig 3 Photograph of EMG from abductor digiti minimi muscle during relaxation Calibration 2 5 m ec and 150 micro V per square Spontaneous activity is observed

Each nerve examined was stimulated on at least two levels (elbow groove and wrist for the ulnar nerve) The laboratory temperature was kept constant

Aldolase and creatine phosphokinase were also examined in 23 patients

RESULTS

The results of the EMG examinations according to the two basic groups of patients appear in table II Of a total of 85 muscles examined 13 (15 3 %) were normal 23 myopathic and 32 polymyositic Of the 35 muscles examined in the non steroid group 6 (17 %) were normal as were 7 (14 %) in the steroid group The difference between these values is too small to have any statistical significance or to indicate any basic difference between the two groups The same may also be said with regard to the pathological findings

Fig 4 Photograph of EMG from anterior tibial muscle during maximal volitional contraction Calibration 15 m sec and 150 micro V per square Loss of motor units prolonged duration and low amplitude are prominent

No significant relation was found between the EMG results and the time lapsed since the onset of disease in either the steroid or non steroid groups It is however of interest that of the 36 muscles examined in the 12 patients suffering from RA for a period of 1—3 years 9 exhibited normal EMG while only 2 out of 21 and 2 out of 15 were normal in the patients suffering for periods of 4—6 and 7—10 years respectively None of the 13 muscles examined in the 5 patients with RA for more than 11 years were normal The results were similar for the steroid and non steroid groups

It would appear that a trend exists towards an inverse relationship between muscles with normal EMG and the duration of disease There also appears to be a tendency towards myopathic and polymyositic EMG recordings in the 1—3 years group which tends to lessen with longer duration of the disease

With regard to the activity of disease at the time of examination only 9 % of the 32 muscles examined in the active group and 19 % of the

TABLE II

Results of EMG in Rheumatoid Arthritis Patients

EMG	Non Steroid	Steroid	Total
Normal	6	7	13
	46 2	53 8	100 0
	17 1	14 0	(15 3)
Myopathy	9	14	23
	39 1	60 9	100 0
	25 7	28 0	(27 1)
Neuropathy	8	9	17
	47 1	52 9	100 0
	22 9	18 0	(0 0)
Polymyositis	12	20	32
	37 5	62 5	100 0
	34 3	40 0	(37 6)
Total	35	50	85
	100 0	100 0	100 0
	(41 2)	(58 8)	100 0

The first line of figures represents the absolute numbers the second line the percentages from the horizontal subtotal the third line the percentages from the vertical subtotal and the figures in brackets the percentages of the subtotals from the total

53 muscles in the inactive group had a normal EMG Most of the 15 muscles with normal EMG belonged to the inactive group Among the abnormal recordings in the active group there was an equal distribution of EMG signs of myopathy and polymyositis while in the inactive group myopathy was found to a markedly lesser degree

The relationship between EMG and location and size of the muscle was next investigated As mentioned above (Table I) EMG was carried out in 56 small intrinsic muscles of the hand and 29 large muscles of the extremities and back Most of the small muscles had shown varying degrees of clinical atrophy in some of them it was difficult to ascertain whether the atrophy was real or only apparent because of the severe deformities of the adjacent joints Their clinical muscle power seemed unaffected except of course for the pain which interfered with the application of resistance

Table III shows the EMG results according to the size of muscles In the small muscles by far the commonest EMG abnormality was of

TABLE III

Relation between EMG and Size of Muscle

EMG		Muscle Small		Muscle Large		Muscle Total	
Normal	Steroid	5	14 3 %	2	17 2 %	7 (8 2 %)	15 3 %
	Non steroid	3		3		6 (7 1 %)	
Myopathy	Steroid	6	21 4 %	8	37 9 %	14 (16 5 %)	27 1 %
	Non steroid	6		3		9 (10 6 %)	
Neuropathy	Steroid	6	23 2 %	3	13 8 %	9 (10 6 %)	20 0 %
	Non steroid	7		1		8 (9 4 %)	
Polymyositis	Steroid	13	41 1 %	7	31 1 %	20 (23 5 %)	37 6 %
	Non steroid	10		2		12 (14 1 %)	
Total		*56*	*100 0 %*	*29*	*100 0 %*	*85*	*100 0 %*

the polymyositic type In the large muscles myopathic changes were pre dominant with slightly less evidence of polymyositis The proportion of EMG abnormalities was about equal in both groups of muscles No marked difference was found between the steroid and non steroid groups

The relation between the results of EMG and the condition of the adjacent joints is presented in table IV No actual relation was found and this impression is corroborated by the fact that approximately the same incidence of abnormal EMG recordings was found both near nor mal and damaged joints regardless of the degree of damage

An additional finding not reflected in the preceding tables is that not one of the 30 patients had normal EMG recordings from all the muscles examined each patient had at least one muscle showing an ab normal EMG It was also remarkable that in many of the patients differ ent muscles had different types of abnormal EMG recordings and in some a variety of EMG patterns were found within the same muscle The latter observation was especially noticeable in the large muscles

CV was examined in 15 ulnar and 3 common peroneal nerves In four ulnar nerves two from patients in the steroid and two in the non steroid group, the velocity and the latency of response were prolonged The delay originated from the area of the elbow and as the joint was

TABLE IV

Relation between EMG and Condition of Adjacent Joints

EMG		Joint damage Normal		Moderate		Mild		Severe		Total	
	Steroid	1		—		3		3		7	
Normal			33 3 %		7 7 %		16 1 %		12 5 %		15 3 %
	Non steroid	2		1		2		1		6	
	Steroid	1		1		4		8		14	
Myopathy			22 3 %		15 3 %		25 8 %		34 4 %		27 1 %
	Non steroid	1		1		4		3		9	
	Steroid	3		2		2		2		9	
Neuropathy			33 3 %		38 5 %		16 1 %		12 5 %		20 0 %
	Non steroid	—		3		3		2		8	
	Steroid	1		3		8		8		20	
Polymyositis			11 1 %		38 5 %		42 0 %		40 6 %		37 6 %
	Non steroid	—		2		5		5		12	
Total		9	100 0	13	100 0	31	100 0	32	100 0	85	100 0
		10 6 %		15 3 %		36 5 %		37 6 %			

found to be damaged in all 4 instances it may be presumed that the pathological finding was caused by an entrapment syndrome

Creatin phosphokinase (CPK) and aldolase were examined in 23 patients 12 of whom were in the non steroid group The CPK was found within normal limits in all of these patients (normal value up to 50 IU) The aldolase (normal value up to 12 IU) was found moderately high in four of the non steroid (18—31 IU) and five of the steroid group (21—42 IU)

CONCLUSIONS

The 85 muscles from 50 patients that were examined by EMG represent too small a number for significant statistical evaluation especially if they are divided into sub-groups The material however has value because it was investigated by a uniform technique with the help of computer analysis and always related to the clinical condition of the patient The results indicate some trends which may be significant

There was practically no difference between the steroid and non steroid groups This implies, at least in our material, that the steroids could not be incriminated as the cause of any EMG abnormality On the other hand it was obvious that RA was the primary cause of muscle or nerve damage in most of the cases Most of the EMG changes observed were either of the polymyositic or myopathic type Only 15 % of the muscles examined showed a normal EMG In this regard it should be stressed that the EMG investigation must be carried out from several points in the muscle as the recording may change radically with only a few millimeters relocation of the electrode

It was of interest that EMG abnormalities were encountered quite early in the course of the disease It should be stressed that in most instances these findings indicated sub clinical damage as with the possible exception of mild atrophy no other neurological signs were present Most of these patients were active It would seem that myopathy is an early sign of RA damage, especially in active patients this is corroborated to a certain extent by the fact that the proportion with myopathy decreases in the inactive cases

The pathological changes are in general ascribed mainly to the small intrinsic muscles of the hand In our material almost the same proportion of both small and large muscles showed abnormal EMG changes mainly of the myopathic or polymyositic type This seeming discrepancy between the clinical picture and EMG findings may possibly be explained by the more obvious signs of atrophy in the small muscles by the fact that in contrast with a large muscle a relatively small number of inflammatory nodules may suffice to cause clinical damage of the small muscle and because in most studies the large muscles were EMG examined from only one or two points if at all

Although it would seem logical to assume that muscles adjacent to damaged joints will show more EMG abnormalities this was not the case in the present material This probably indicates that the muscle damage is primary and not related to an inflammatory process spreading from the synovia to the muscle such an occurrence is not however impossible especially in the small muscles

In no patient with RA regardless of the duration or activity of the disease were all the muscles found to have a normal EMG This suggests that the muscles in RA are damaged more often than one suspects the damage however may be subclinical and detectable only by a careful extensive EMG investigation

With regard to the EMG examination it is our impression that in seeking mild widely located pathological changes, only a very extensive search may demonstrate their presence The use of computer analysis will help in the measurement of duration parameters and this may prove of cardinal importance in the detection of borderline changes

In four instances the motor conduction velocity demonstrated the presence of a probable entrapment syndrome This finding was obviously related to a damaged joint with resultant compression of the nerve It is however of interest that clinical signs were not present in any of these cases

The enzyme investigations were of no value Aldolase which is by far a more sensitive enzyme was found in abnormal quantities in 9 of the 23 patients This may however be related to causes other than muscular disorders since the CPK which is a better indicator of muscle damage was normal in all the cases

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THE PREVALENCE OF RHEUMATOID ARTHRITIS IN DIFFERENT GEOGRAPHICAL AREAS IN SWEDEN

By

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Summary In the general population of 39 418 persons within areas in the counties of Norrbotten (BD) Jamtland (Z) Skaraborg (R), Kristianstad west coast (LV), and Kristianstad east coast (L) in Sweden all the inhabitants over the age of seven (in Norrbotten over the age of 14) were investigated for the total number of cases of rheumatoid arthritis Re investigations of non response (10—20 %) were made The southernmost and northernmost areas were about 1,500 km apart Every person was examined naked or seminaked The diagnostic criteria prepared by the American Rheumatism Association (ARA) were used In the mathematical calculations it was possible to determine indices for correct comparisons of the prevalences in the different areas

The prevalence for RA (classical definite probable, possible) in the populations examined was 3 1 per cent

The highest figures were observed in the southern part of the country (L LV) The prevalences were higher in the coastal areas (in L LV and BD) than in the inland areas (Z, R) The prevalence was higher in the non response group than in the primarily investigated group

This survey was designed to determine the prevalence of rheumatoid arthritis (RA) according to the ARA criteria in five different geographical areas in Sweden involving altogether total populations of 39 418 individual persons investigated The intention was also to make comparisons between the different areas and to get as exact comparison as possible

MATERIAL AND METHODS

A detailed account of the medical aspects of this study has been given elsewhere (1, 21)

Populations investigated

A *Total general populations* over the age of seven were examined as to the prevalence of RA in the following five major regions in the counties of (a) Norrbotten (BD) 8 897 persons (3 916 males and 4 981 females) (b) Jämtland (Z) 3 302 persons (1 662 males and 1 640 females) (c) Skaraborg (R) 10 465 persons (5 258 males and 5 207 females) (d) Kristianstad west coast (LV) 7 382 persons (3 613 males and 3 769 females) (e) Kristianstad east coast (L) 5 287 persons (2 571 males and 2 716 females)

B *Special populations* included (a) Steel workers in a steel works in Norrbotten 1 298 males (b) Prisoners in the Open Borstal Institution of Ulriksfors Jamtland 99 males (c) Males undergoing medical examination on enlistment in the enrolment area of Io 3 (parts of the counties of Örebro and Vastmanland) 825 males (d) Males in the defence forces refresher training (Revinge and Hassleholm Kristianstad west) 721 males (e) Re investigation in Norrbotten and Kristianstad 1 142 persons (482 males and 660 females)

Diagnostic criteria The diagnosis of RA was based on case notes and macromorphological objective findings from joints muscles and tendons often completed with data from hospital records and x ray documents Use was made of the diagnostic criteria prepared by the American Rheumatism Association (ARA) (46) and esp the criteria for the population studies (28) Definitions see (21) The history of RA included family history past history course of the disease previous hospital care x ray results Noted were joint pain type and duration morning stiffness effect of rest and exercise on joint pain the distribution of joint involvement type of spread symmetry of involvement signs of a systematic reaction subcutaneous nodules Joint examination technique see reference (21)

Sampling methods Total sampling was used in the primary investigations of persons over the age of seven (Norrbotten over the age of 14) purposive selection among children under this age systematic random sampling for the groups (10—20 %) not responding to the call for the primary investigation Be and for the group designed Bc above

TABLE I

The Prevalence of Rheumatoid Arthritis (Classical Definite Probable and Possible) in Randomly Selected and Investigated Persons among the Non response Group and among Randomly Matched Persons from the Primary Survey

N R = the non response group
P I = the primarily investigated group

Area in	Total no investigated	Classical N R	Classical P I	Definite N R	Definite P I	Probable N R	Probable P I	Possible N R	Possible P I
Norrbotten	353	1	0	10	1	3	2	3	3
Kristianstad, west and east coast	789	6	0	11	5	15	5	14	5
Total	1 142	7	0	21	6	18	7	17	8

Investigation routine Every person within the areas passed seminaked or naked through an examination room Here their joints were investigated (21) Data on sex age name occupation birth civil status address etc of all the individuals in the populations were ascertained from up to date registers of the population The initial investigation in some respects was a form of sorting out the healthy from the rheumatoid arthritics Supplementary investigations on rheumatoid arthritics were then performed during some hours extra each day

Non response generally varied between 10—15—20 % By random systematic sampling among the non response group in Norrbotten and Kristianstad every sixth and every fourth person respectively was investigated Those in the non response group being investigated were individually matched with persons of the same sex age and from the same geographical area but who had been investigated in the primary survey The prevalences of RA in the two groups were then compared (Table I) It could be shown that the prevalences of RA (classical definite probable possible) were higher in the non response group than in the primarily investigated group The reasons for this non response are discussed in (21)

Reliability and *validity* of the investigations could be considered as good The diagnoses in the primary investigations were often checked with the help of hospital records The training factor in the investigations was eliminated in a primary pilot study The comparisons had a high accuracy as all the investigations were made by the same investigator

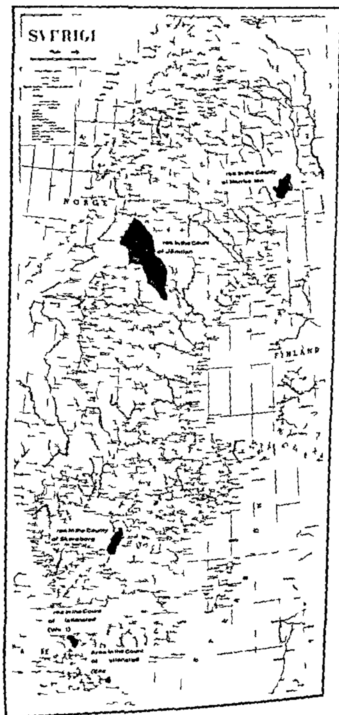

SVERIGE
NORGE
FINLAND

TABLE II

Percentage of a) Classical RA b) Definite RA c) Probable RA and d) Possible RA in the Counties of Norrbotten Jamtland Skaraborg and Kristianstad (west and east coast)

Standardized indices where disturbing influences by differences in age distribution and occupational distribution between the areas have been eliminated The index values are calculated such as to be used for "vertical" comparisons primarily in the tables

Area in	No investigated	No RA	Per cent	Stand index Age eliminated	Stand index Occupation eliminated
a)					
Norrbotten	10 506	30	0 29	101 86	82 39
Jamtland	3 333	7	0 21	91 30	100 60
Skaraborg	11 197	15	0 13	49 74	51 24
Kristianstad west	7 798	34	0 44	153 58	158 69
Kristianstad east	5 836	19	0 33	128 63	1 0 71
b)		No RB			
Norrbotten	10 506	66	0 63	86 75	86 29
Jamtland	3 333	21	0 63	106 06	127 90
Skaraborg	11 197	91	0 81	121 99	121 63
Kristianstad west	7 798	46	0 39	8[illegible] 34	85 88
Kristianstad east	5 836	41	0 [illegible]0	108 24	103 31
c)		No RI			
Norrbotten	10 506	89	0 85	85 46	83 [illegible]1
Jamtland	3 333	19	0 57	72 45	75 12
Skaraborg	11 197	77	0 69	75 88	73 62
Kristianstad west	7 798	79	1 01	106 75	106 69
Kristianstad east	5 836	94	1 61	181 29	18[illegible] 38
d)		No RT			
Norrbotten	10 506	147	1 40	100 69	97 06
Jamtland	3 333	28	0 84	75 22	84 43
Skaraborg	11 197	140	1 [illegible]5	97 88	97 74
Kristianstad west	7 798	[illegible]9	1 01	77 04	5 84
Kristianstad east	5 836	109	1 87	149 18	154 29

RA = classical rheumatoid arthritis
RB = definite rheumatoid arthritis
RI = probable rheumatoid arthritis
RT = possible rheumatoid art

Hospital and out patient records and x ray data were collected for the populations investigated and they helped to establish the primary diagnoses for RA

Time schedules Primary investigations alternatively in the northernmost and southernmost areas Skaraborg January to May 1961 Jamtland May to June 1961 Norrbotten January to May 1962 Kristianstad west and east coast May to June 1962 *Re investigations in Norrbotten* April 1963 in *Kristianstad* May 1963

Geography The distance between the northernmost and southernmost areas is about 1 500 km A precise account of the geography climate geology vegetation and water supply of the areas in which the populations live is given elsewhere (21) Approximately the areas where the investigations were performed in Norrbotten was 65°26 N 4°07 E Jamtland 63°50 N 2°28 E Skaraborg 58°26 N 3°53 E Kristianstad west 56°26 N 5°12 E Kristianstad east 55°34 N 3°43 E

RESULTS

The prevalence of RA (classical definite probable possible) in general total populations in different geographical areas is shown in table II

The standard indices in table II have been calculated during elimination of age and occupational differences It is clear that Kristianstad west coast has the highest prevalence and Skaraborg by far the lowest The index and percentage values agree on the whole indicating that age and occupational differences between the areas do not have any significant effect in this case

When ranking the geographical areas with consideration to the prevalence of RA from that with the lowest to that with the highest prevalence the results were *Classical* R Z BD L LV *Definite* LV BD Z L R *Probable* Z R BD LV L *Possible* Z LV R BD L

Table III shows the corresponding information taking sex differences into account The higher prevalence of RA among females is apparent

The highest prevalences of RA are found in the coastal areas especially in the county of Kristianstad but also in Norrbotten The lowest prevalences are found in the inland (Jamtland and Skaraborg)

It is a well known fact that the prevalence of RA in different geographical areas in the world varies (23) Even if the climatic environ

TABLE III

Prevalence and Standardized Indices for a) Classical RA b) Definite RA c) Probable RA and d) Possible RA in Areas Investigated Males and Females Separated

Possible disturbing influences from differences in age distribution and occupational distribution between areas are eliminated in the indices Indices primarily comparable in vertical direction in the tables

	Males				Females			
Area in	No RA	Per cent	Stand index Age eliminated	Stand index Occupation eliminated	No RA	Per cent	Stand index Age eliminated	Stand index Occupation eliminated
a)	No RA				No RA			
Norrbotten	8	0 15	73 13	50 97	22	0 42	117 50	99 92
Jamtland	4	0 23	129 25	151 81	3	0 18	66 96	66 54
Skaraborg	5	0 08	41 54	43 34	10	0 19	54 66	57 67
Kristianstad [illegible]	8	0 37	171 24	189 86	20	0 50	140 80	138 39
Kristianstad east	14	0 47	141 49	175 85	11	0 39	116 67	170 37
b)	No RB				No RB			
Norrbotten	20	0 38	87 56	81 37	46	0 88	87 08	89 81
Jamtland	6	0 35	90 74	171 70	15	0 9	117 78	132 87
Skaraborg	34	0 57	140 78	140 07	57	1 10	114 11	113 6
Kristianstad west	14	0 37	80 73	90 08	3	0 80	81 63	83 07
Kristianstad east	10	0 34	87 49	65 5	31	1 09	119 31	120 00
c)	No RI				No RI			
Norrbotten	4	0 45	68 75	59 43	65	1 24	91 30	93 84
Jamtland	6	0 35	65 71	64 84	13	0 80	78 74	77 56
Skaraborg	19	0 37	55 77	54 9	58	1 17	86 70	87 49
Kristianstad west	29	0 77	1 395	178 36	50	1 24	96 71	90 99
Kristianstad east	38	1 8	6 77	3 31	56	1 96	161 43	16 60
d)	No RT				No RT			
Norrbotten	47	0 89	89 33	81 39	100	1 91	107 06	105 66
Jamtland	8	0 47	55 38	75 7	0	1 73	90 30	90 64
Skaraborg	54	0 90	90 44	101 67	80	1 66	98 30	90 3
Kristianstad west	31	0 8	85 41	85 61	49	1 19	71 17	[illegible]
Kristianstad east	44	1 48	165 56	161 76	63	[illegible] 9	141 52	[illegible]

RA = classical rheumatoid arthritis
RB = definite rheumatoid arthritis
RI = probable rheuma[illegible]
RT = possible rheum[illegible]

TABLE IV

The Prevalence of RA in the Special Population Investigated All Males

	Prevalence		
	Classical + Definite RA	Probable RA	Possible RA
Steel workers (BD)	0 4 %	0 7 %	1 4 %
Prisoner (Z)			1 0 %
Males age 18 on enlistment duty			1 9 %
Refresher training soldiers	0 7 %	1 1 %	

ment has not been shown to influence the prevalence and severity of joint diseases it does appear to influence the complaint threshold (27) That the prevalence of RA seems to be related to climatic factors is mainly based on reports suggesting a low prevalence in some tropical countries e g Liberia (30 38) Many patients state that they are adversely affected by cold and wet weather (23 51) No country or race has so far been shown to be free from RA except the 259 inhabitants of Tristan da Cunha (4)

Some prevalences of RA reported from different parts of the world shall be given here Due to differences in the age distribution the figures in the present series are however not comparable with those in the literature In a *report to be published* where age prevalences of RA in the present series will be accounted for more correct comparisons with the figures in the literature will be made

Europe

The geographic incidence of RA seems to be rather uniform *in* North Western Europe (35) *Sweden* In 1943 the prevalence of RA in some populations in Sweden was shown to be 2 0 per cent for both sexes (15 16) females 2 7 % males 1 3 % (50) The figures were based on field studies performed by a staff of especially trained medical students In another report from 1943 it was stated that 2 5 per cent of the population of Sweden had to see a doctor for their RA (6 a) Rheumatic diseases in Sweden have been estimated to 7 4 % (6 b 53) *Denmark* In 1932 0 8 per thousand and 3 000—4 000 new cases every year (3) In 1945 the prevalence of RA was estimated to be 0 67 % (20) and 0 3 % in 1945

(43) *Greenland* Eskimos 0 3 % over he age of 5 yrs (17) *Finland* Heinola (definite & probable) females 10 2 % males 4 4 % over the age of 25 (33) In another report the arthritis was estimated to be 0 9 % females 0 3 % males (24) *Norway* The number of RA invalids perhaps amounts to 10 000 (Hegna quoted in 29) *England* Wensleydale (definite & probable) 9 0 % females 2 0 % males age 55—64 (19) Leigh and Wensleydale (definite & probable) 3 7 % females 2 0 % males over the age of 25 (31, 36) Leigh (definite & probable) females 17 7 % 5 6 % males age 55—64 yrs (19) Rhondda Wales 2 1 % males 34—64 yrs of age (37) 8 9 % females 35—64 yrs (19) Glamorgan Wales 8 1 % females 35—64 yrs of age (19) *Ireland northern* The prevalence of RA was the same as in England (32) *Netherlands* In Schiermonnikoog (definite & probable) 1 6 % females 1 3 % males (13) Rotterdam (definite & probable) 12 6 % females 3 6 % males age group 55—64 yrs (19) *Czechoslovakia* Piestany (definite & probable) 1 5 % females 0 6 % males over the age of 25 (52) *Italy* The prevalence has been reported to be rather low (42)

U S A & Canada

Alaskan Eskimos (definite & probable) 9 1 % females 0 % males over 20 yrs of age (5) Haida Indians Canada (definite & probable) 2 5 % females 1 8 % males over the age of 25 (19 45) Blackfeet Indians Montana (definite & probable) 5 0 % females 3 9 % males over the age of 35 (7 8) Tecumseh Michigan (definite & probable) 2 7 % females 0 6 % males over the age of 20 (41) Pittsburgh (definite & probable) females 7 1 % males 0 7 % over the age of 35 (10)

Central & South America

Puerto Rico (definite & probable) females 2 2 % males 0 5 % over the age of 35 (40) *Jamaica* (definite & probable) females 13 2 % males 6 8 % age groups 35—64 yrs (37) *Chile* females 9 1 % males 5 5 % (39)

Asia & Pacific

Israel (definite & probable RA) 2 0 % females 0 5 % males over the age of 20 (2) *Liberia* Very few cases among the natives but 12 3 per cent of the native population showed positive sheep cell agglutination tests (38)

Japan (definite & probable RA) Osaka region 0 7 % females 0 1 % males over the age of 20 (49)

New Zealand Maoris (definite & probable) 4 0 % females 3 8 % males over the age of 20 (47 48)

New Guinea The prevalence is low and no new cases among 15 000 persons (14)

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ALPHA MANNOSIDASE ACTIVITY IN SYNOVIAL FLUID*

By

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Summary A microassay technique for the evaluation of synovial fluid alpha mannosidase activity is presented with the evaluation of 181 synovial fluid samples from a variety of arthropathic conditions The results indicate a close correlation of enzyme activity with synovial fluid cell count and the degree of inflammation associated with the various types of joint disease It is suggested that the evaluation of this and other lysosomal glycosidases offer a quantitative method for the evaluation of inflammatory joint disease

Since the original description of lysosomes and lysosomal enzymes by de Duve and his associates in 1955 (5) a great deal of interest has developed in these subcellular organelles and their relationship to the inflammatory process Over the years many investigators have studied their properties in order to elucidate the role of these hydrolytic enzymes in inflammatory joint disease The present study was undertaken to evaluate alpha mannosidase activity one of the lysosomal glycosidases in the synovial fluid from various arthropathic conditions The purpose of these studies is to establish a reproducible microassay for this enzyme activity and a correlation between various connective tissue diseases synovial fluid cell count and alpha mannosidase activity

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* This study was supported by USPHS grant no 94677 and a grant from Ge ST Pharmaceuticals

MATERIALS AND METHODS

Synovial fluids were obtained from 184 patients suffering from a variety of rheumatic diseases Only those fluids received in the laboratory within 90 minutes of aspiration from patients having a single definite clinical diagnosis were included in the study Synovial fluid white cell counts were performed and the specimen was frozen and stored for enzymatic assay

The alpha mannosidase activity was based on the release of p nitrophenol from p nitrophenyl alpha mannopyranoside (Sigma Chemical Company St Louis Missouri) under optimal incubation conditions discussed under results The incubations were rendered basic with sodium carbonate and diluted to one milliliter with the optical density determined at 400 millimicrons in a Beckman DU 2 spectrophotometer The assay is based on the formation of a chromophore by the free p nitrophenol under basic conditions This reaction does not occur with the intact p-nitrophenyl glycoside

RESULTS

The development of a reproducible microassay for alpha mannosidase activity was paramount to its evaluation in synovial fluid samples from various disease entities Using this assay method it was determined as shown in fig 1 that substrate saturation was readily accomplished at an eight micromole per milliliter level With p-nitrophenyl alpha mannoside as the substrate, the K_m value of the synovial fluid alpha mannosidase activity was $1.55 \times 10^{-3}M$ as determined from fig 1 and shown superimposed on the substrate saturation curve and plotted as [s]/V versus [s] according to the method of Lineweaver and Burke (7) The optimal buffer requirements were determined to be a sodium acetate acetic acid buffer pH 4 0 No metal ion requirement was demonstrated and the alpha mannosidase activity was not influenced by the addition of varying amounts of EDTA (sodium versonate) The enzyme activity was not appreciably altered by repeated freezing and thawing for a period of at least 21 days and was linear with respect to incubation time for a minimal time period of two hours Fig 2 illustrates the linear response of alpha mannosidase activity to increasing amounts of synovial fluid and indicates synovial fluid volumes of five to forty m

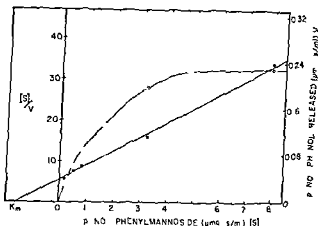


Fig 1 Substrate saturation curve and K_m determination (o) represents the substrate saturation curve with V expressing micromoles of p nitrophenol released per milliliter of incubation mixture per hour of incubation and [s] expressing substrate concentration in micromoles of p nitrophenyl mannoside per milliliter of incubation mixture (•) represents the K_m plot with the K_m intercept at 1 55 x 10^{-3}M

liters may be used as necessary for quantitation The routine synovial fluid assay contains in a final volume of 125 µliters 1 0 µmole of p nitrophenyl alpha mannoside 25 µmoles sodium acetate acetic acid buff

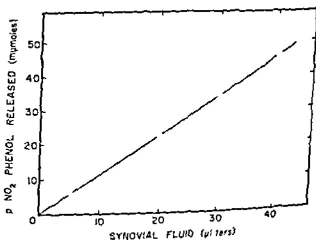


Fig 2 Enzyme concentration curve The incubation conditions are as described in the text with variable amounts of synovial fluid used as the enzyme source and product formation is expressed as *millimicromoles of p nitrophenol released per incubation mixture*

er, pH 4 0, and 10—40 μliters of synovial fluid This mixture is then incubated at 37°C for 90 minutes

Following standardization of the microassay method for quantitation of alpha mannosidase activity 184 synovial fluid samples were evaluated for *enzyme activity* In table I *the results are categorized according* to disease entity It is obvious that a wide range of enzyme activity exists in any one group of patients and that some of the groups are too small for individual statistical significance It is apparent from the average values that the non inflammatory arthritides (degenerative joint disease and post traumatic arthritis) have low synovial fluid alpha mannosidase activity On the other hand the *truly inflammatory arthropathies* (Reiter s syndrome and below in table I) exhibit high levels of synovial fluid enzyme activity The very wide range observed in the patients suffering from RA appears to be related to disease activity with the highest levels found in patients with severe proliferative synovitis and rapid bone destruction In contrast the lower levels are seen in patients whose dis

TABLE I

Alpha Mannosidase Activity in Various Arthritic Conditions

	Specimens	Alpha mannosidase* range of activity	Alpha mannosidase* average activity
Normal	5	5?—≤15	22
Degenerative joint disease	21	. 7— 15	63
Post traumatic arthritis	6	131— 34	70
Ankylosing spondylitis	4	04— 97	144
Reiter s syndrome	5	655— 209	364
Rheumatoid arthritis** (Adul)	113	1650— 31	393
Rheumatoid arthritis (Juvenile)	7	1540— 74	395
Pseudogout* •	15	1450— 68	489
Gout**	10	2030— 148	591

* Alpha mannosidase activity is expressed as millimicromoles of p nitrophenol released per milliliter of synovial fluid per hour of incubation

** All patients fulfilled the Amer Rheum Assoc criteria for classical definite or probable rheumatoid arthritis

• Demonstration of the appropriate intracellular crystals under compensated polarized light was required for these two diagnoses

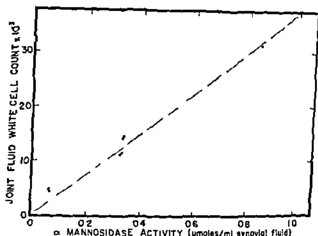


Fig 3 Correlation of synovial fluid white cell count and alpha mannosida e activity from patients with *RA* *Cell count is expressed as cells per* mm^3 *x* 10^{-3} *and* enzyme activity as µmoles of p nitrophenol released per ml of synovial *fluid* per hour of incubation

ease is minimal and whose major clinical problems are often of a mechanical nature The highest levels of enzyme activity are seen in those disease entities classified as crystalline arthropathies acute gout and pseudogout

A comparison of synovial fluid alpha mannosidase activity with the commonly used index of joint inflammation the synovial *fluid white* cell count, is illustrated in fig 3 This figure shows the relationship between these two parameters in patients suffering *from RA* A close but imperfect relationship is obvious which has a *correlation coefficient* of 0 75 On the other hand if this same *relationship is illustrated for* the twenty three patients having gout or pseudogout fig 4 is a med at demonstrating an even closer relationship *between synovial fluid* white blood cell count and enzyme activity *The correlation coefficient* in this case is 0 98 supporting the contention *of an even closer relation*ship in this type of inflammatory arthropathy

DISCUSSION

The lysosomal acid hydrolases are well accepted as contributing *factors* in the perpetuation of chronic inflammatory processes *The demonstra*

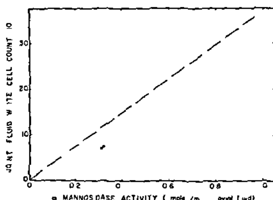


Fig 4 Correlation of synovial fluid white cell count and alpha mannosidase activity from patients with gout and pseudogout Cell count is expressed as cells per mm³ x 10^{-3} and enzyme activity as μmoles of p nitrophenol released per ml of synovial fluid per hour of incubation (o) represents patients with pseudogout and (•) represents those with gout

tion of a number of these enzymes in inflammatory synovial fluid has been previously reported by various investigators One group of the acid hydrolases can be classified as lysosomal glycosidases Some of the en zyme activities represented in this group have been previously studied in synovial fluid Examples of which are beta N acetylglucosaminidase (2 3) and beta glucuronidase (6) In addition some comparative stud ies have been carried out correlating various lysosomal enzyme activities in synovial fluid samples Examples of this type of study are the com parison of acid phosphatase and beta N acetylglucosaminidase activity (4) and the correlation of beta N acetylglucosaminidase activity beta glucu ronidase and beta galactosidase activity (1) in synovial fluid All these studies suggest a correlation of lysosomal glycosidase activity with the presence and severity of inflammatory joint disease The present study offers a simple microassay technique for an additional lysosomal glyco sidase not previously studied in synovial fluid The data presented shows a close correlation between alpha mannosidase activity and the type and degree of joint disease present If synovial fluid cell count is accepted as one parameter of severity in the evaluation of inflammatory joint disease alpha mannosidase activity offers another quantitative means of evaluation

The role that this particular enzyme activity may have in the perpetuation of the degradative phase of inflammatory joint disease remains obscure However one cannot help but speculate on the necessity of the presence of an alpha mannosidase in the catabolism of the carbohydrate side chains of at least serum glycoproteins The recent description of mannosidosis (8) establishes a clinical entity associated with a hereditary absence of lysosomal alpha mannosidase activity resulting in the tissue accumulation of mannose rich oligosaccharides In contrast it seems logical that the inappropriate and excessive release of this enzyme in inflammatory arthritides could lead to the alteration or degradation of connective tissue or exudative glycoproteins

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SCANNING ELECTRON MICROSCOPIC STUDIES OF EXTENSOR TENDON DEGENERATION IN RHEUMATOID ARTHRITIS

By

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Summary Examination of the surfaces of four normal and six rheumatoid tendons in the hand by scanning electron microscopy clearly showed the processes of degeneration affecting the tendons in rheumatoid arthritis These findings seemed to correspond to the clinical state

The surfaces of the healthy tendons displayed fine networks of closely enmeshed collagen fibrils and parallel arrangements of collagen bundles In acute rheumatoid tenosynovitis numerous inflammatory cells were found on the surfaces of the tendons later crater and groove formations followed as was observed in degenerated tendons and ruptured stumps in cases in which the active stage already had calmed down

The hypothesis is discussed that disorganization of tendons in rheumatoid disease may be caused by lysosomal enzymes present in greater concentration in the synovial fluid components of rheumatoid patients than in healthy subjects

Several possibilities have been presented as a cause of rheumatoid tendon ruptures 1 Invasion of the tendon by rheumatoid granulation tissue 2 Vascular obliteration (7) 3 Attrition by bony spicules (6 12 13) 4 Pressure of the weakened flexor tendons against the carpal ligament (10) 5 Result of local steroid injections (8)

Combination of causes 1 and 3 may also occur (4)

In order to further elucidate the pathogenesis of tendon changes in RA the authors have studied the surface of six degenerated or ruptured tendons with scanning electron microscope (SM) Four normal tendons served as control

Recently there have been reports of SEM observations of the joint cartilage and synovial membrane in healthy and pathological states (2 3 5, 11) However the authors have not found any descriptions of the surface structure of the tendons studied by SEM

MATERIAL AND METHODS

The normal specimens were obtained from the tendons of ext carpi rad at amputation of an upper extremity for a sarcoma in the humerus (female 21 year old) and from the ext dig comm of two autopsy cases following cardiac infarction (both males 61 and 64 year-old) One control sample of ext poll long tendon was taken from a 5? year old drowned male

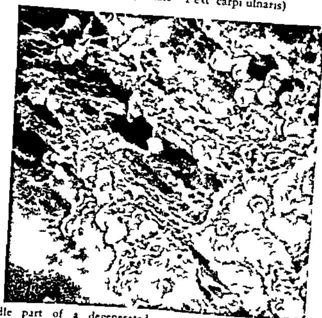
Our material of the ruptured and degenerated tendons consists of six patients suffering from definite RA according to the criteria of ARA

Clinically the material can be divided into two groups Group I in which the tendons were surrounded by large tenosynovitic masses and fluid containing rice bodies and Group II with low grade inflammation Patients L M and R R did not have a rupture of the examined tendons but the tendon of the ext dig min propr was degenerated elongated and attached to surrounding tissue over a distance of 3 cm distal to the site of the ulnar heads which in the case E M was earlier removed Thus any attrition effect was excluded All patients belonging to the Group II gave a history of swelling at the site where the rupture later took place

The dehydrated (2 5) specimens were examined either by a JSM 2 type scanning electron microscope (Japan Electron Ltd) or Stereoscan instrument (Cambridge Instrument Company) The electron micrographs at low magnifications of 60 demonstrated the general patterns of the surfaces which corresponded to those seen by binocular light microscopy According to the gross findings the specimens without shedding and distortion were examined in higher magnifications under scanning electron microscopy



Fig 1 Normal tendon x 1 000 (female 71 ext carpi ulnaris)



g Middle part of a degenerated ext dig V proprius tendon x 1 000 A t e tenosynovitis



Fig 3 Border of degenerated and healthy tendon, × 3 000 Rupture of ext comm dig IV distal stump Active tenosynovitis

RESULTS

Healthy tendon surface The micrographs both at lower and higher magnifications demonstrated similar patterns in the surface structure composed of large bundles measuring 5—6 μ in diameter and fine networks interwoven with fibrils of thickness of 0 1—0 2 μ (Fig. 1) According to the thickness the former appeared to correspond to the collagen bundles running longitudinally in the tendon and the latter to the collagen fibrils recognized in transmission electron microscopy Occasionally some small collagen bundles measuring 1—2 μ in diameter were found lying transversely In general, the healthy tendon surfaces showed an even smooth structure where most of the fibrillar components were glued to each other with interfibrillar ground matrix

Rheumatoid tendon surface The tendon specimens from the rheumatoid patients in the two clinical groups already mentioned were examined Group I The surface of the ext dig V propr from the

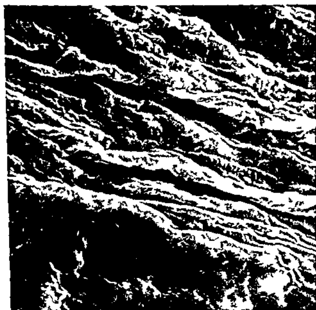


Fig 4 Middle part of a degenerated ext dig V proprius tendon x 1 000 Low grade inflammation

patient E M revealed many inflammatory cells ranging 5—6 μ in diameter a marked unevenness due to cavity formations and splitting of the fibrillar components (Fig 2) In another case where the tenosynovitis was recognized macroscopically the surface of the tendon stump showed similar changes with the appearance of the inflammatory cells of 5—6 μ as mentioned above and irregular ridges formed by splitting of collagen bundles (Fig 3)

Group II The surface of the mid part of the degenerated and elongated but not ruptured tendon from the patient R R showed irregular crater and groove formations lying parallel to the direction of the collagen bundles Inflammatory cells were not detected (Fig 4) In higher magnification from another case (B L) belonging to this group it was confirmed that the collagen bundles and fibrils were exposed on the uneven surfaces probably due to the dissolution of the ground matrix (Fig 5)

Ruptured stump The surfaces at the ends of the ruptured rheumatoid tendons displayed marked disorganization showing splitting and



Fig 5 Rupture of ext poll longus border of healthy and degenerated tendon. x 3 000 Low grade inflammation

curling of the collagen bundles (Fig 6) and a large number of craters and hollows

DISCUSSION

Three dimensional views of the synovial joints have already provided interesting material in the biophysiological study of the human joints as well as in their pathological changes The present work has indicated that study of the fine surface configuration of healthy and rheumatoid tendons is valuable towards the understanding of the pathogenesis of rheumatoid tendon degeneration and rupture

The surface structure of healthy tendons seems to be of interest in the study of functions of the synovial systems Scanning electron micrographs of the healthy tendon surfaces clearly showed that the collagen bundles and the fine networks enmeshed by collagen fibrils are of regular pattern It is suggested that these surfaces are covered with



Fig 6 Ruptured end of the ext poll long x 1 000 *Mild inflammation*

lubricating synovial fluid in the tendon sheath cavity and this film may protect the tendons from attrition Surface structure of the tendon seems to be very similar to that of joint cartilage (2 3 5 11) but the direction of collagen bundles is different It is also supposed that both tissues are similarly placed in the synovial system

On the other hand the surface changes in rheumatoid disease showed the disorganization of the affected tendons Particularly many inflammatory cells appeared on the surface of the tendons which had been surrounded by tenosynovitic masses It has been shown earlier by one of the authors (H I) that numerous inflammatory cells are found on the synovial membranes of the rheumatoid joints as in the joint fluid

This implies that those cells on the rheumatoid tendons originate from the surrounding inflammatory tissues Such cells in rheumatoid synovial fluid may contain many proteolytic enzymes acting on the connective tissue polysaccharides Especially hydrolytic enzymes released from lysosomes in rheumatoid joint fluid cells have been supposed to play an important role in the inflammation of rheumatoid disease (14)

Recently it was found that the activity of muramidase one of such hydrolytic enzymes is elevated in the rheumatoid synovial fluid (9) Fell and Dingle showed that lysosomal enzymes were capable of breaking down the proteinpolysaccharide of cartilage matrix (1)

It is suggested that the tendons of rheumatoid patients are degenerated by the action of lysosomal enzymes The present results demonstrated many crater and hollow formations and many uneven undulations formed by denatured fibrillar components Similar changes have been observed in rheumatoid joint articular cartilage (2 3 11)

These observations may imply that the released lysosomal enzymes primarily dissolve the ground matrix and the exposed collagen bundles and fibrils are then subjected to collagenases It may be postulated that such degenerated tendons in rheumatoid patients can spontaneously rupture even without mechanical attrition

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FIBRIN DISSOLUTION IN SYNOVIAL FLUID

By

R BACH ANDERSEN and JOHS GORMSEN

Summary Fibrinogen fibrinogen fibrin breakdown products (b d p) fibrin stabilizing factor (FSF) plasminogen alpha 2 macroglobulin and alpha 1 antitrypsin concentrations were estimated immunologically in normal and in pathological synovial fluids from patients with RA *gout monoarthritis rheumatic fever, osteoarthritis and traumatic* arthritis The fibrinolytic activity was determined by the fibrinplate method

Normal synovial fluid contained no fibrinogen b d p FSF or alpha 2 macroglobulin but traces of plasminogen and alpha 1 anti trypsin By increasing inflammatory reactions increasing amounts of the various factors were demonstrable No distinct specific patterns were found

Spontaneous *fibrinolytic activity was demonstrable in the most ex* sudative cases and in these high concentrations of fibrin breakdown products were present These were also demonstrable in most patholog *ical synovial fluids* proving a recent proteolytic activity

It is suggested that the fibrin breakdown products in pathological synovial fluids are partly resistent to proteolytic activity

It is suggested that fibrin persisting within joints might act as an antigenic pool and provide a permanent stimulus to antibody formation (10 12 14 27) Fibrinogen is not found in normal synovial fl

(15, 29), from which it is rapidly cleared after injury (17) Fibrinogen is present in various concentrations in inflammatory arthritis and especially in RA and tuberculous arthritis is precipitated as fibrin (12) Some of this is degradated by proteolytic enzymes as plasmin Fibrinolytic activity of synovial fluid has been reported (7 10 12 26), and degradation products of fibrinogen fibrin were demonstrated in pathological synovial fluid by Schur & Sanderson (30) and Barnhart et al (10) Some fibrin might be digested by cellular proteases (10 11 22) and some phagocytized by exsudative neutrophiles (28) Fibrin might *remain partly incorporated within inflammatory* material as in *Rice* Bodies (1)

Synovial fluid a complex mixture of substances derived from plasma contains also products from cells in synovial membranes and from extra*vascular leucocytes As far as other clotting factors is concerned* normal synovial fluid contains fc XI and XII very little plasminogen (21) and some antiproteolytic activity (2, 20) whereas pathological synovial fluids might contain fc II V VIII and antithrombin (10)

Plasminogen activator activity very little thromboplastic activity (9) and fibrin stabilizing factor (FSF) activity (5) has been demonstrated in synovial membranes and cartilage by itself might also activate plasminogen (22)

The aim of the present work was to quantitate fibrinolytic activity of synovial fluids from normal individuals and patients suffering from various kinds of joint diseases to quantitate and differentiate immunologically some factors of decisive importance for clearing of fibrin as fibrinogen plasminogen alpha 1 antitrypsin alpha 2 macroglobulin and fibrin stabilizing factor (FSF) and to quantitate the fibrin degradation products by sensitive and specific hemagglutination inhibition immunoassays as such informations are lacking Similar parameters *in plasma* were investigated in order to elucidate the relationship between local or systemical reactions

MATERIALS AND METHODS

Clinical material the patients studied were selected at *random* among in and out patients *Inflammatory cases* (Table I) this group comprises 26 with classical and 5 with definite RA *according* to the criteria from the American Rheumatism Association (4) *furthermore* 3 patients

TABLE I

Inflammatory Joint Diseases

Clinical Data

Diagnosis	Cases	Sex		Age		Lansbury's clin index			ESR		A 2 glob	Duration in months	Sero-reactions	
		f	m	mean	range	cases	mean	range	range	mean	mean	range	pos	neg
RA Group I	13	11	2	60	48—70	8	108	86—134	85	78—91	1 0	1/4—1)	9	3
RA Group II	12	7	5	53	17—79	5	66	60— 71	64	38—90	1 06	2/3—15	5	5
RA Group III	6	2	4	60	49—67	5	50	50	26	14—35	0 9	1/2—44	2	2
Juvenile	3		1	11	9—12	—	—	—	28	16—30	—	2— 4	0	3
Gout	5	0	5	51	31—76	—	—	—	52	7—11	0 9	—16	0	5
Monoarthritis	7	0	7	42	26—60	—	—	—	8	2—14	0 9	10	0	6
Rh fever	2	0	2	37	21—54	—	—	—	122	120—125	1 0	(	1	1

with juvenile RA, 5 with gout (UA) 9 with monoarthritis (MA) including 2 with rheumatic fever (RF) The patients with RA were divided into three subgroups in respect to the activity of their disease determined clinically in 18 patients according to Lansbury s clinical index (23), except for gripstrength and in 15 by erythrocyte sedimentation rate (ESR Westergren) and serum alpha 2 globulin (estimated in g% agarelectrophoresis) in agreement with Lansbury s clinical index (23) *Group I* high activity clinical index more than 80 or ESR more than 70 mm/hour and alpha 2 globulin more than 1 g% *Group II* middle activity clinical index 50—80 or ESR 35—70 mm/hour alpha 2 globulin 0 9—1 % *Group III* no activity clinical index below 50 ESR below 35 mm/hour and alpha 2 globulin less than 0 9 g% *The non inflammatory cases* comprise 7 patients with osteoarthrosis (OA) (5 men 2 women average age 57 years range 27—73 years average ESR 20 mm/hour range 9—25 mm/hour) and 9 patients suspected for tears of the meniscus (TA) (16 men and 3 women average age 28 years range 18—49 years average duration of the symptoms 12 months duration 1—60 months ESR range 2—14 mm/hour) *Pathological synovial fluids* were obtained by joint puncture 10—60 ml from each *Normal synovial fluids* were obtained by aspiration of knee joints in 15 persons without joint diseases (age 25—60 years) in amounts of 0 1—0 5 ml in each This material was pooled In all cases nine parts of synovial fluid were added to one part of trisodium citrate with or without EACA (final concentration 10^{-2} M) The supernatants after centrifugation at 3 000 r p m for 10 min at room temperature were withdrawn and stored at —20°C Synovial fluids were in some cases heated at 60 C for 30 min for control experiments *White cell counts* were performed in 30 cases (Table II) *fibrinagar electrophoresis* in 13 patients with RA and in 3 with OA 5 with MA and in 8 with TA *Plasma samples* were collected from 32 patients 17 with RA 3 with UA 7 with TA and 5 with OA were anticoagulated and centrifuged as described above A *reference plasma* was obtained by pooling citrated platelet poor plasma from 10 healthy individuals This was kept at —20°C in small fractions *Antisera* anti alpha 2 macroglobulin anti alpha 1 antitrypsin and antiplasminogen were obtained from Behring Werke A G Marburg Lahn *Fibrinogen* lyophilized human fibrinogen grade L 96 % clottable contaminated by plasminogen and TSF from AB KABI Stockholm *Bovine thrombin* Behring Werke A G Marburg Lahn stock solution 20 units/ml *Urokinase©* human prep-

TABLE II

Leucocyte Counts and Albumin/Globulin Ratio in Synovial Fluids

iagnosis	Cases	Leucocyte court per µl mean	range	Albumin%/Globulin% mean	range
A Group I	7	14 445	(4 800—22 400)	39/6	(32—46/54—68)
A Group II	8	9 000	(2 500—25 600)	44/56	(34—56/44—66)
A Group III	3	2 100	(500— 3 800)	54/46	(51—56/44—49)
iout	4	7 000	(1 870—11 840)	49/51	(44—53/47—56)
Ionoarthritis	1	5 640		54/46	
Isteoarthritis	4	450	(0— 670)	62/38	(55—68/32—45)
'raumatic arthritis	3	160	(0— 200)	—	

ration from Leo Pharmaceutical Products Ballerup Denmark Fresh dilutions 600 units/ml *Plasmin* Lysofibrin(R) A/S NOVO Industry Copenhagen fresh dilution 10 C U/ml *Agar* Noble Difco Laboratories Detroit Michigan USA *Hyaluronidase* Hyalase 20 000 units/ml from Leo Halsingborg Sweden A stock solution 1 000 units/ml of MC Ilvain s buffer (phosphate-citricacid buffer pH 7 0 M 0 15)

A mixture of 0 6 ml of synovial fluid and 0 1 ml of the hyalase stock solution was kept overnight at 37°C By this procedure the viscosity is reduced to the viscosity of distilled water Merthiolate (1 10 000) was added to avoid unspecific bacterial fibrinolytic activity

METHODS

Fibrin plate method was carried out according to the principles of Astrup & Mullertz (8) in the modification of Alkjaersig et al (3) Heated fibrin plates 85 C for 30 min The lytic activity was estimated as spontaneous activity and after incubating aspirates with 600 units of urokinase/ml at 37°C for 15 min

Single radial immunodiffusion was carried out according to the principles described by Mancini (24) Ten ml of 3 per cent W/v agarsolution in 0 1 M sodium barbital buffer (pH 8 6) and 10 ml of 0 9 % (W/V) chloride containing the specific antiserum were mixed at 50 C and transferred into the space between two glass plates giving a 1 mm

TABLE III

Amounts of Special Proteins in Reference Plasma Given in Square mm Determined by Radial Immunodiffusion a m Mancini

Ref plasma	Fibrinogen*)	FSF	Plasminogen	A1 antitrypsin	A macro globulin
100 %	39 4±5 7	27 9±3 6	26 5±4 2	66 1±10 3	33 4±4 4
50 %	25 5±3 1	20 9±2 4	16 6±1 7	38 6± 6 2	22 8±2 8
25 %	16 5±2 5	15 1±1 5	12 4±1 9	23 5± 0 8	16 4±3 4
12 5 %	12 3±0 9	10 3±2 1	9 0±0 6	19 3± 2 8	17 9±7 6

*) Fibrinogen both fibrinogen and breakdown products are measured

thick layer of agar (Merthiolate 1 10 000) kept in a moist chamber and *used the same day Exactly 2 µl of the material to be investigated were* applicated in holes of 2 mm of diameter All testings were carried out in duplo Incubation period 48 hours at room temperature for fc XIII estimation 72 hours Dilutions (12 5 % 25 % 50 % 100 %) of the reference plasma (with known fibrinogen concentration) were tested on every plate The areas of the precipitates are given in square mm The dilution curve of the reference plasma gave a straight line expressed semilogarithmically up to the concentration of 50 % Higher concentrations gave a slight curve formation Aspirates exhibiting higher activities than 50 % were diluted for further investigations Standard deviations for estimations of the actual factors of the reference plasma measured on seven different plates are given in table III The spread of 350 determinations of the various factors had a variation of less than 5 % The concentrations of the various factors were given in percentages read on the daily standard curve The concentrations of fibrinogen were also calculated in mg% The amounts of antisera were 0 5 ml antiplasminogen 0 5 ml antifibrinogen 1 0 ml anti alpha 2 macroglobulin 1 3 ml anti FSF and 5 ml of alpha 1 antitrypsin per plate

Fibrinogen fibrin breakdown products = b d p = split products were determined by hemagglutination *inhibition immunoassays* (H I I A) according to the technique of Merskey et al (25) as described elsewhere (15) H I I A were carried out before and after adding thrombin 50 i u /ml of synovial fluid

Fibrin agar electrophoresis was performed according to the principles of Heimburger and Schwick (18 19) using a 2 7 per cent agar in a

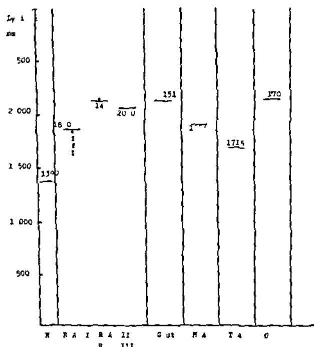


Fig 1 Urokinase induced fibrinolytic activity of synovial fluids measured on fibrin plates Normal fluid 1 390 square mm Also the mean values for the various pathological groups are given in the figure

diethyl barbitural acetate buffer pH 8 2 ionic strength 0 05 6 volt/cm for 45 min at room temperature Thrombin was added in a concentration of 0 2 ml (20 units/ml) Final fibrinogen concentration 100 mg%

No macroscopic hemolysis was seen in any synovial fluid examined Nearly all pathological synovial fluids contained some precipitates partly consisting of fibrinogen fibrin which were unsoluble by heating the sample at 37°C

RESULTS

Fibrin plate Method

The normal pooled synovial fluid and synovial fluids from non inflammatory cases exhibited no spontaneous activity Eight of 31 syno-

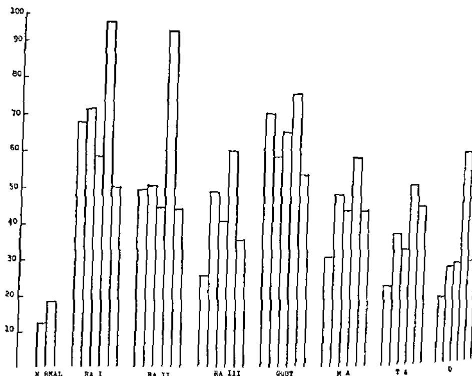


Fig 2 Radial immunodiffusion the relative concentrations (mean) of the various factors are illustrated Fibrinogen means fibrinogen including b d p Normal fluid contains no fibrinogen split products FSF or alpha 2 macroglobulin but traces of plasminogen and alpha 1 antitrypsin In each group the columns illustrate 1 = fibrinogen 2 = FSF 3 = plasminogen 4 = alpha 1 antitrypsin 5 = alpha 2 macroglobulin

vial fluids from the RA group all from RA I showed spontaneous activity (150—289 mm²) on unheated plates and very little on heated plates Urokinase activated fluids (Fig 1) from RA patients and some other inflammatory cases showed significantly higher lytic activity than normal synovial fluid possibly due to a higher content of plasminogen — see later None of the plasma samples showed any spontaneous activity

Radial Immunodiffusion

The concentrations of fibrinogen (including split products) plasminogen FSF alpha 1 antitrypsin and alpha 2 macroglobulin in the total material are illustrated by fig 2

TABLE IV

Fibrinogen antigenic Fractions in Synovial Fluid in µg/ml Determined by H I I A before and after Thrombin Addition

Diagnosis	No thrombin			After thrombin	
	cases	mean	range	mean	range
RA Group I	4	265	143— 358	265	143— 358
RA Group II	3	645	143—1 433*)	573	143—1 433
RA Group III	3	214	35— 573	112	17— 286
Gout	1	716		716	
Traumatic arthritis	4	398	17— 716	183	17— 386
Osteoarthritis	2	107	71— 143	107	71— 143

*) One patient had extremely high concentration 1 433 µg/ml

Normal synovial fluid contained no fibrinogen split products fc XIII or alpha 2 macroglobulin whereas small amounts of plasminogen and alpha 1 antitrypsin were traced by the techniques used

Pathological Synovial Fluids

Fibrinogen the amounts of fibrinogen antigenic proteins found in synovial fluids are listed in table II Highest concentrations were seen in RA group I and II and in gout i e in cases with most pronounced inflammatory reactions (Tables I and II) The experiments with H I I A (Table IV) showed that most of the fibrinogen antigenic proteins were breakdown products unclottable by thrombin as the amounts found after thrombin addition still were high and even might remain un changed dealing with pronounced inflammatory fluids

Plasminogen the mean percentage in the total patient group was 45 In general highest concentrations were found in RA group I (58 5) decreasing in group II (44 5) and group III (41 5) In UA the per centage was 55 5 Lowest concentrations were found in OA

FSF the mean percentage in the patient group was 45 Highest con centrations were found in RA group I and II and in UA whereas the percentages in group OA were between mean and zero

Alpha 1 antitrypsin the mean percentage of the patient group was 65 Increased concentrations were especially seen in RA group I (95 0) decreasing to 93 0 in group II and to 60 5 in group III The other groups were close to mean and between mean and zero

TABLE V

Fibrinogen antigenic Fractions in mg% in Synovial Fluid Plasma Determined by Radial Immunodiffusion a m Mancini

Diagnosis	Synovial fluid			Plasma		
	cases	mean	range	cases	mean	range
RA Group I	9	216	54—604	4	399	360 0—444
RA Group II	9	132	51—456	6	271	14? 5—43?
RA Group III	5	79 5	33—16?	?	?83	?43 0—3?4
Gout	3	212	105—300	3	3 4	?17 5—4?6
Monoarthritis	7	93	?2—291	1	375	
Traumatic arthritis	18	65	0—150	6	?12	1 6 0—300
Osteoarthritis	5	56	24— 66	4	344	187 0—4?6

Alpha 2 macroglobulin average percentage of the whole group was 43 Increased concentrations were especially found in RA group I (51 %) and in UA and the lowest was in group OA

Correlation between synovial fluid and plasma concentrations of fibrinogen antigenic fractions estimated by radial immunodiffusion were in all patients significantly higher in plasma The differences between concentrations in plasma and synovial fluid were most pronounced in

TABLE VI

Ratio between Amounts of Various Factors in Synovial Fluid and Plasma

Diagnosis	Fibrinogen*)		FSF		Plasminogen		A1 antitrypsin		A2 macroglobulin	
	cases	ratio	cases	ratio	cases	ratio	cases	ratio	cases	ratio
RA Group I	4	0 30	7	0 61	7	0 54	7	0 60	5	0 64
RA Group II	6	0 34	8	0 44	8	0 47	6	0 70	8	0 68
RA Group III	2	0 11	2	0 27	2	0 39	?	0 60	2	0 6
Gout	3	0 34	3	0 7?	3	0 47	3	0 69	3	0 65
Traumatic arthritis	5	0 32	7	0 31	7	0 37	7	0 45	6	0 68
Osteoarthritis	5	0 06	5	0 53	5	0 55	5	0 69	5	0 57

*) Fibrinogen means fibrinogen antigenic fractions fibrinogen and fibrinogen degradation products

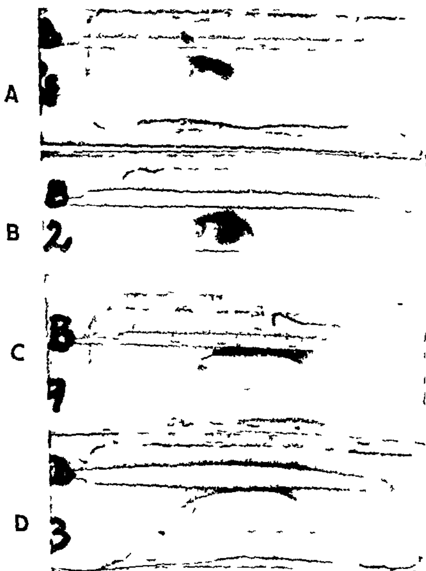


Fig 3 Fil n aga immunoelect ophoresis

A non inflammatory synovial fluid in center hole Anti alpha 2 macroglobulin in top well and plasmin in bottom well.

B as A but inflammatory synovial fluid in center hole

C non inflammatory synovial fluid in top well and plasmin in bottom well

D as C, but inflammatory synovial fluid in center hole Cathode right

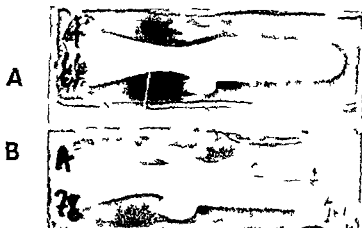


Fig 4 Fibrin agar immunoelectrophoresis

A non inflammatory *synovial fluid* in bottom hole and plasma from the same individual in top hole Plasmin in centre well
B inflammatory synovial fluid in bottom hole plasma *from the same patient* in top hole Plasmin in center well

less inflammatory cases (Table V) The fibrinogen antigenic fractions in synovial fluid from inflammatory cases consisted especially of non clottable *fibrinogen fractions* The real fibrinogen ratio is consequently lower than listed in the table The ratio between concentrations in synovial fluid and plasma of the other factors (Table VI) were highest *dealing with* pronounced inflammatory reactions but without any specific parameters

Fibrin agar electrophoresis plasminogen moves towards the anode alpha 1 antitrypsin towards *the cathode* whereas *alpha 2 macroglobulin* and fibrinogen only exhibit mobility The plasmin inhibition of alpha 2 macroglobulin appeared more pronounced in inflammatory than in non inflammatory fluids but *exact quantitation* was *not* possible (Fig 3) Alpha 1 antitrypsin showed very little if any inhibitory effect and the precipitin lines appeared comparable dealing with inflammatory and non inflammatory *synovial fluids* *The more* pronounced antiplasmin effect in synovial fluid in inflammatory cases was also reflected in plasma (Fig 4)

Some spontaneous lytic activity was generally seen cathodal to the origin (Figs 3 and 4) also if tested on heated fibrin plates *If the* synovial fluid was heated at 60°C for 30 min before *application no* spontaneous lytic activity *was seen*

Effect of hyaluronic acid incubation of synovial fluid with hyaluronidase did not influence any of the results obtained by radial immunodiffusion or on fibrinolytic activity after incubation with urokinase

DISCUSSION

The experiments dealt with in the present paper were especially focused on proteins influencing dissolution and consequent removement of fibrin precipitates in inflammatory synovial fluids This fibrin might be important for perpetuating inflammatory reactions As fibrinogen fibrin generally is cleared rapidly from normal synovial fluid distinct biochemical reactions might be responsible for the persistence of fibrin in pathological cases

Fibrinogen fibrin is normally degradated by plasmin into two soluble non clottable plasmin resistant fractions (ref 15) with molecular weight of around 88 000 and 56 000 Other proteolytic enzymes might if present in the inflammatory synovial fluid split fibrin further to peptides easier removable

Spontaneous fibrinolytic activity was significant in synovial fluids from patients with active RA That proteolytic activity also had been significant in most cases was illustrated by the fact that high concentrations of fibrinogen fibrin breakdown products were demonstrated in these by HIIA

The persistence of fibrin in spite of proteolytic activity might be due to one or several reasons The high concentrations of plasmin inhibitors as alpha 2 macroglobulin and alpha 1 antitrypsin especially in pronounced inflammatory fluids might be of significance These inhibitors might also inhibit other proteases if present The fibrin stabilizing factor found in synovial membranes (5) and in synovial fluids in inflammatory cases might accelerate the stabilization of fibrin Stabilization by itself does not however make fibrin more resistant to plasmin activity (16) More likely highmolecular fibrin breakdown products form complexes with gammaglobulins mucopolysaccharides and possibly with other factors (22) *and that these complexes become resistant against proteolysis by plasmin*

A further characterization of the fibrin breakdown products and studies on other protease activities in inflammatory synovial fluids are seriously needed

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